



VERIFICATION OF TRANSLATION

I, Masaaki IWABUCHI, 1-1-301, Kaigan 1-chome, Minato-ku, Tokyo 105-0022 Japan, state the following:

I am fluent in both English and Japanese languages and capable of translating documents from one into the other of these languages.

The attached document is a true and accurate English translation to the best of my knowledge and belief of:

Japanese Patent Application No. H11(1999)-027710

I state that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true.

Signature : Masaaki IWABUCHI

Masaaki IWABUCHI

Date : July 4, 2006

[Designation of Document] Patent Application
[Reference Number] A99017
[Filing Date] February 4, 1999
[Address] Commissioner of the Patent Office
[International Classification] C12N 15/10
[Title of the Invention] Novel G Protein Coupled Receptor Protein and its DNA
[Number of Claim(s)] 26

[Inventor]
[Address] Takeda Kasuga Haitsu 404,7-9, Kasuga 1-chome,
Tsukuba-shi, IBARAKI
[Name] WATANABE Takuya

[Inventor]
[Address] 10-1-909-303, Namiki 4-chome, Tsukuba-shi, IBARAKI
[Name] TERAO Yasuko

[Inventor]
[Address] Takeda Kasuga Haitsu 703,7-9, Kasuga 1-chome,
Tsukuba-shi, IBARAKI
[Name] SHINTANI Yasushi

[Applicant for patent]
[Identification Number] 000002934
[Name Person or Legal Person] TAKEDA CHEMICAL INDUSTRIES, LTD.
[Representative] TAKEDA Kunio

[Attorney]
[Identification Number] 100073955
[Patent Attorney]
[Name Person or Legal Person] ASAHIINA Tadao

[Appointed Attorney]
[Identification Number] 100110456
[Patent Attorney]
[Name of Person or Legal Person] UCHIYAMA Tsutomu

[Priority Based on Prior Application]
[Application Number] 10-305949
[Filing Date] October 27, 1998



11(1999)-027710

[Reference Number]

A98206

[Details of Commission]

[Prepayment Register Number] 005142

[Amount of Payment] 21000

[List of What It Submitted]

[Name of What Is Submitted] Specification 1

[Name of What Is Submitted] Drawings 1

[Name of What Is Submitted] Abstract 1

[Number of General Power of Attorney] 9000053

[Number of General Power of Attorney] 9721047

[Request of poof] Yes



[Document] SPECIFICATION

[Title of the invention]

Novel G Protein COUPLED Receptor Protein and its DNA

5

[Claims]

1. A G protein coupled receptor protein and salts thereof comprising the same or substantially the same amino acid sequence as the amino acid sequence shown by SEQ ID NO: 1.
2. A protein according to claim 1, in which substantially the same amino acid sequence is represented by SEQ ID NO: 5.
3. A partial peptide of the G protein coupled receptor protein according to claim 1 or salts thereof.
4. A polynucleotide containing a polynucleotide having the base sequence encoding the G protein coupled receptor protein according to claim 1.
5. A polynucleotide according to claim 4, which is DNA.
6. A polynucleotide according to claim 4, which contains the base sequence represented by SEQ ID NO: 2 or SEQ ID NO: 6.
7. A recombinant vector containing the polynucleotide according to claim 4.
8. A transformant transformed by the recombinant vector according to claim 7.
9. A method for manufacturing the G protein coupled receptor protein or salts thereof according to claim 1, which comprises culturing the transformant according to claim 8 and producing the G protein coupled receptor protein according to claim 1.
10. An antibody to the G protein coupled receptor protein according to claim 1, the partial peptide according to claim 3 or salts thereof.

35

11. An antibody according to claim 10 which is a neutralizing antibody to inactivate signal transduction of the G protein coupled receptor protein according to claim 1.

5 12. A diagnostic composition comprising the antibody according to claim 10.

10 13. A ligand to the G protein coupled receptor protein or salts thereof according to claim 1, which is obtainable using the G protein coupled receptor protein according to claim 1, the partial peptide according to claim 3 or salts thereof.

14. A pharmaceutical composition comprising the ligand to the G protein coupled receptor according to claim 13.

15 15. A method for determining the ligand to the G protein coupled receptor protein or salts thereof according to claim 1, wherein the G protein coupled receptor protein according to claim 1, the partial peptide according to claim 3 or salts thereof are used.

20 16. A method for screening a compound or salts thereof that alter the binding property between a ligand and the G protein coupled receptor protein or salts thereof according to claim 1, which comprises using the G protein coupled receptor protein according to claim 1, the partial peptide according to claim 3 or salts thereof.

30 17. A kit for screening a compound or salts thereof that alter the binding property between a ligand and a G protein coupled receptor protein or salts thereof according to claim 1, comprising the G protein coupled receptor protein or salts thereof according to claim 1, a partial peptide according to claim 3 or salts thereof.

35 18. A compound or salts thereof that alter the binding property between a ligand and the G protein

coupled receptor protein or salts thereof according to claim 1, which is obtainable using the screening method according to claim 16 or the screening kit according to claim 17.

5 19. A pharmaceutical composition comprising the compound or salts thereof that alter the binding property between a ligand and the G protein coupled receptor protein or salts thereof according to claim 1, which is obtainable using the screening method
10 according to claim 16 or the screening kit according to claim 17.

20. A polynucleotide that hybridizes with the polynucleotide according to claim 4 under high stringent conditions.

15 21. A polynucleotide comprising complementary base sequence to the polynucleotide according to claim 4 or a part thereof.

20 22. A method for quantifying mRNA of the receptor protein according to claim 1, which comprises using the polynucleotide according to claim 4 or a part thereof.

23. A method for quantifying the receptor protein according to claim 1, which comprises using the antibody according to claim 10.

25 24. A diagnostic agent of disorders related to the function of the G protein coupled receptor protein according to claim 1, which comprises using the quantifying methods according to claims 22 or 23.

30 25. A method for screening the compound or salts thereof that alter the expression of the G protein coupled receptor according to claim 1, which comprises using the quantifying methods according to claims 22 or 23.

26. A compound or salts thereof that alter the expression of the G protein coupled receptor according

to claim 1, which is obtainable using the screening method according to claim 25.

[Detailed description of the invention]

5 [0001]

[FIELD OF THE INVENTION]

The present invention relates to a novel G protein coupled receptor protein derived from the rat cerebellum and human brain, or salts thereof and DNA 10 encoding the same.

[0002]

[BACKGROUND ART]

A variety of hormones and neurotransmitters 15 regulate the functions in vivo through specific receptor proteins located in a cell membrane. Many of these receptor proteins mediate the transmission of intracellular signals via activation of guanine nucleotide-binding proteins (hereinafter sometimes referred to as G proteins) with which the receptor is 20 coupled. These receptor proteins possess the common structure, i.e. seven transmembrane domains and are thus collectively referred to as G protein coupled receptors or seven-transmembrane receptors (7TMR).

G protein coupled receptor proteins exist on each functional cell surface of cells and internal organs of a living body and play very important roles as the targets of molecules, for example, hormones, neurotransmitters, physiologically active substances 30 and the like, which molecules control, regulate or adjust the functions of cells and internal organs in the living body. These receptor proteins mediate signal transduction in a cell by binding to physiologically active substances and various reactions such as 35 activation or inhibition of cells is caused by the thus transmitted signal.

To clarify the relation between substances which regulate complex functions in cells and internal organs of various living bodies and their specific receptor proteins, in particular, G protein coupled receptor 5 proteins, would elucidate the functional mechanisms of cells and internal organs in various living body and thus provide a very important means for development of drugs having close relation to such functional mechanisms.

10

[0003]

For example, in various organs of a living body, the physiological functions are controlled through regulation by many hormones, hormone-like substances, 15 neurotransmitters or physiologically active substances. In particular, physiologically active substances present in various sites of a living body regulate its physiological functions through each of the corresponding receptor proteins. Still, there are many 20 unknown hormones, neurotransmitters or other physiologically active substances exist in vivo and only a few receptor proteins have been reported on their structures so far. In addition, it yet remains unclear if there will be subtypes of known receptor 25 proteins.

It is also very important for development of pharmaceuticals to clarify the relation between substances that regulate complex functions in vivo and their specific receptor proteins. Furthermore, for 30 efficient screening of agonists and antagonists to receptor proteins in development of pharmaceuticals, it was necessary to elucidate the functions of receptor protein genes expressed in vivo and to express the genes in an appropriate expression system.

In recent years, random analysis of cDNA sequences has been actively performed as a method for analyzing genes expressed in vivo. The sequences of cDNA fragments thus obtained have been registered and 5 published to databases as Expressed Sequence Tag (EST). However, since most EST contains only the sequence information, the function is predictable only with difficulty.

10 [0004]

[Problems to be solved]

Substances that inhibit the binding of G protein coupled proteins to physiological active substances (i.e., ligands) and substances that bind to 15 physiologically active substances thereby to induce signal transductions similar to those induced by the physiologically active substances (i.e., ligands) have been used for pharmaceuticals as antagonists or agonists specific to the receptors for regulating the 20 biological functions. Accordingly, it is very important to discover a new G protein coupled receptor protein that is not only important for physiological expression in vivo but can be a target for developing pharmaceuticals and to clone the genes (e.g., cDNA), in 25 search for a specific ligand, agonist, and antagonist of the novel G protein coupled receptor.

However, not all G protein coupled receptors have been found. Even now, there are many unknown G protein coupled receptors and those for which the corresponding 30 ligands are unidentified, that is, orphan receptors. It has thus been seriously awaited to explore a novel G protein coupled receptor and clarify its function.

G Protein coupled receptors are useful in searching for a novel physiologically active substance 35 (i.e., ligand) using the signal transduction activity

as an index and in searching for agonists and antagonists of the receptor. Even if no physiological ligand is found, agonists and antagonist of the receptor may be prepared by analyzing the physiological activity of the receptor through receptor inactivation experiments (knockout animal). Ligands, agonists, and antagonists of the receptor are expected to be used as prophylactic/therapeutic and diagnostic drugs for diseases associated with dysfunction of the G protein coupled receptor.

Hypofunction or hyperfunction of the G protein coupled receptor due to genetic variation of the receptor in vivo causes some disorders in many cases. In this case, the G protein coupled receptor may be used not only for administration of antagonists or agonists of the receptor, but also for gene therapy by transfer of the receptor gene into the body (or certain specific organs) or by transfer of the antisense nucleic acid to the receptor gene. In such a gene therapy, information on the base sequence of the receptor gene is essentially required for searching of deletion or mutation in the gene. The receptor gene is also applicable as prophylactic/therapeutic and diagnostic drugs for diseases associated with dysfunction of the receptor.

The present invention provides a novel and useful G protein coupled receptor protein as described above. That is, the present invention provides a novel G protein coupled receptor protein, its partial peptides and salts thereof, as well as polynucleotides (DNA and RNA, and derivatives thereof) containing polynucleotides (DNA and RNA, and derivatives thereof) encoding the G protein coupled receptor protein and its partial peptides, recombinant vectors containing the polynucleotides, transformants bearing the recombinant

vectors, methods for manufacturing the G protein coupled receptor protein and salts thereof, antibodies to the G protein coupled receptor protein, its partial peptides and salts thereof, compounds that alter the expression level of said G protein coupled receptor protein, methods for determination of ligands to the G protein coupled receptor protein, methods for screening compounds (antagonists and agonists) and salts thereof that alter the binding property of ligands and the G protein coupled receptor protein, kits for use in the screening method, compounds (antagonists and agonists) or salts thereof that alter the binding property of ligands obtainable by the screening method or obtainable using the screening kit and the G protein coupled receptor protein, and pharmaceutical compositions comprising the compounds (antagonists and agonists) that alter the binding property of ligands to the G protein coupled receptor protein, or compounds or salts thereof that alter the expression level of the G protein coupled receptor protein.

[0005]

[Means for solving the problems]

The inventors performed extensive studies and as a result, succeeded in isolation of cDNA encoding the novel G protein coupled receptor protein derived from the rat cerebellum and human brain, based on the EST information prepared by the degenerated PCR method, resulting in successful analysis of the entire base sequence of the cDNA. The amino acid sequence deduced from the base sequence has supported that the first to the seventh transmembrane domains were observed on the hydrophobic plotting analysis, confirming that the protein encoded by the cDNA is a transmembrane G protein coupled receptor protein passing through the

membrane seven times. Based on these findings, the present inventors made extensive studies and as a result, the present invention has been accomplished.

5 [0006]

Thus, the present invention relates to:

(1) A G protein coupled receptor protein and salts thereof comprising the same or substantially the same amino acid sequence as the amino acid sequence shown by
10 SEQ ID NO: 1.

(2) A protein according to (1), in which substantially the same amino acid sequence is represented by SEQ ID NO: 5.

15 (3) A partial peptide of the G protein coupled receptor protein according to (1) or salts thereof.

(4) A polynucleotide containing a polynucleotide having the base sequence encoding the G protein coupled receptor protein according to (1).

20 (5) A polynucleotide according to (4), which is DNA.

(6) A polynucleotide according to (4), which contains the base sequence represented by SEQ ID NO: 2 or SEQ ID NO: 6.

25 (7) A recombinant vector containing the polynucleotide according to (4).

(8) A transformant transformed by the recombinant vector according to (7).

30 (9) A method for manufacturing the G protein coupled receptor protein or salts thereof according to (1), which comprises culturing the transformant according to (8) and producing the G protein coupled receptor protein according to (1).

[0007]

(10) An antibody to the G protein coupled receptor protein according to (1), the partial peptide according to (3) or salts thereof.

5 (11) An antibody according to (10) which is a neutralizing antibody to inactivate signal transduction of the G protein coupled receptor protein according to (1).

(12) A diagnostic composition comprising the antibody according to (10).

10 (13) A ligand to the G protein coupled receptor protein or salts thereof according to (1), which is obtainable using the G protein coupled receptor protein according to (1), the partial peptide according to (3) or salts thereof.

15 (14) A pharmaceutical composition comprising the ligand to the G protein coupled receptor according to (13).

20 (15) A method for determining the ligand to the G protein coupled receptor protein or salts thereof according to (1), wherein the G protein coupled receptor protein according to (1), the partial peptide according to (3) or salts thereof are used.

25 (16) A method for screening a compound or salts thereof that alter the binding property between a ligand and the G protein coupled receptor protein or salts thereof according to (1), which comprises using the G protein coupled receptor protein according to (1), the partial peptide according to (3) or salts thereof.

30 (17) A kit for screening a compound or salts thereof that alter the binding property between a ligand and a G protein coupled receptor protein or salts thereof according to (1), comprising the G protein coupled receptor protein or salts thereof according to (1), a partial peptide according to (3) or
35 salts thereof.

(18) A compound or salts thereof that alter the binding property between a ligand and the G protein coupled receptor protein or salts thereof according to (1), which is obtainable using the screening method 5 according to (16) or the screening kit according to (17).

(19) A pharmaceutical composition comprising the compound or salts thereof that alter the binding property between a ligand and the G protein coupled 10 receptor protein or salts thereof according to (1), which is obtainable using the screening method according to (16) or the screening kit according to (17).

(20) A polynucleotide that hybridizes with the 15 polynucleotide according to (4) under high stringent conditions.

(21) A polynucleotide comprising complementary base sequence to the polynucleotide according to (4) or a part thereof.

20 (22) A method for quantifying mRNA of the receptor protein according to (1), which comprises using the polynucleotide according to (4) or a part thereof.

(23) A method for quantifying the receptor protein according to (1), which comprises using the antibody 25 according to (10).

(24) A diagnostic agent of disorders related to the function of the G protein coupled receptor protein according to (1), which comprises using the quantifying methods according to (22) or (23).

30 (25) A method for screening the compound or salts thereof that alter the expression of the G protein coupled receptor according to (1), which comprises using the quantifying methods according to (22) or (23).

(26) A compound or salts thereof that alter the 35 expression of the G protein coupled receptor according

to (1), which is obtainable using the screening method according to (25).

[0008]

5 The present invention further provides:

(27) A G protein coupled receptor protein or salts thereof according to (1) comprising: (i) an amino acid sequence represented by SEQ ID NO: 1 in which one, two, or more amino acids (preferably 1 to 30 amino acids, 10 more preferably 1 to 9 amino acids, most preferably several amino acids) are deleted; (ii) an amino acid sequence represented by SEQ ID NO: 1, to which one, two, or more amino acids (preferably 1 to 30 amino acids, more preferably 1 to 10 amino acids, most preferably 15 several amino acids) are added; (iii) an amino acid sequence represented by SEQ ID NO: 1, in which one, two, or more amino acids (preferably 1 to 30 amino acids, more preferably 1 to 10 amino acids, and most preferably several amino acids) are substituted by 20 other amino acids; and (iv) a combination of the above amino acid sequences;

(28) A method for determining the ligand according to (15), which comprises contacting a test compound with the G protein coupled receptor protein or salts 25 thereof according to (1) or the partial peptide according to (2) or salts thereof.

(29) A method for determining the ligand according to (27), which includes angiotensin, bombesin, canavinoïd, cholecystokinin, glutamine, serotonin, 30 melatonin, neuropeptide Y, opioid, purines, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedulin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, vasoactive intestinal polypeptide (VIP), somatostatin, dopamine, motilin, amylin, bradykinin, calcitonin gene-35 related peptide (CGRP), leukotrienes, pancreastatin,

prostaglandins, thromboxane, adenosine, adrenaline, α and β -chemokines (e.g., IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP-1 α , MIP-1 β , RANTES, etc.), endothelin,
5 enterogastrin, histamin, neuropeptid Y, TRH, pancreatic polypeptide or galanin,

[0009]

(30) A method for screening according to (16),
10 wherein comparison is made between (i) the case where the G protein coupled receptor protein or salts thereof according to (1) or the partial peptide or salts thereof according to (3) are brought in contact with the ligand and (ii) the case where the G protein
15 coupled receptor protein or salts thereof according to (1) or the partial peptide or salts thereof according to (3) are brought in contact with the ligand and a test compound;

(31) A method for screening a compound or salts thereof that alter the binding property between a ligand and the G protein coupled receptor protein or salts thereof according to (1), which comprises measuring and comparing (i) an amount of labeled ligand bound to the G protein coupled receptor protein or salts thereof according to (1) or the partial peptide or salts thereof according to (3) where the labeled ligand is brought in contact with the G protein coupled receptor protein or salts thereof according to (1) or the partial peptide or salts thereof according to (3)
25 and (ii) an amount of labeled ligand bound to the G protein coupled receptor protein or salts thereof according to (1) or the partial peptide or salts thereof according to (3) where the labeled ligand and a test compound are brought in contact with the G protein
30 coupled receptor protein or salts thereof according to (1) or the partial peptide or salts thereof according to (3) where the labeled ligand and a test compound are brought in contact with the G protein
35 coupled receptor protein or salts thereof according to (1) or the partial peptide or salts thereof according to (3)

(1) or the partial peptide or salts thereof according to (3);

(32) A method for screening a compound or salts thereof that alter the binding property between a
5 ligand and the G protein coupled receptor protein or salts thereof according to (1), which comprises measuring and comparing (i) an amount of labeled ligand bound to cells containing the G protein coupled receptor protein according to (1) upon bringing the
10 labeled ligand in contact with the cells, and (ii) an amount of labeled ligand bound to the cells upon bringing the labeled ligand and a test compound in contact with the cells;

(33) A method for screening a compound or salts thereof that alter the binding property between a ligand and the G protein coupled receptor protein or salts thereof according to (1), which comprises measuring and comparing (i) an amount of labeled ligand bound to a membrane fraction of cells containing the G
20 protein coupled receptor protein according to (1) upon bringing the labeled ligand in contact with the cell membrane fraction, and (ii) an amount of labeled ligand bound to a membrane fraction of the cells upon bringing the labeled ligand and a test compound in contact with
25 the cell membrane fraction;

[0010]

(34) A method for screening a compound or salts thereof that alter the binding property between a
30 ligand and the G protein coupled receptor protein or salts thereof according to (1), which comprises measuring and comparing (i) an amount of labeled ligand bound to a G protein coupled receptor protein expressed in cell membrane of said transformant of (8) by
35 culturing the transformant where the labeled ligand is

brought in contact with the G protein coupled receptor protein expressed, and (ii) an amount of labeled ligand bound to a protein expressed in cell membrane of said transformant (8) by culturing the transformant where
5 the labeled ligand and a test compound are brought in contact with the G protein coupled receptor protein expressed;

(35) A method for screening a compound or salts thereof that alter the binding property between a
10 ligand and the G protein coupled receptor protein or salts thereof according to (1), which comprises measuring and comparing (i) a G protein coupled receptor protein-mediated cell stimulating activity wherein a compound that activates the G protein coupled
15 receptor protein or salts thereof according to (1) is brought in contact with cells containing the G protein coupled receptor protein according to (1) and (ii) a G protein coupled receptor protein-mediated cell stimulating activity wherein a compound that activates
20 the G protein coupled receptor protein or salts thereof according to (1) and a test compound are brought in contact with the cells containing the G protein coupled receptor protein according to (1);

(36) A method for screening a compound or salts thereof that alter the binding property between a ligand and the G protein coupled receptor protein or salts thereof according to (1), which comprises measuring and comparing (i) a G protein coupled receptor protein-mediated cell stimulating activity
25 wherein a compound that activates the G protein coupled receptor protein or salts thereof according to (1) is brought in contact with a G protein coupled receptor protein expressed in a cell membrane of the
30 transformant according to (8) by culturing the transformant and (ii) a G protein coupled receptor
35

protein-mediated cell stimulating activity wherein a compound that activates the G protein coupled receptor protein or salts thereof according to (1) and a test compound are brought in contact with a G protein
5 coupled receptor protein expressed in a cell membrane of the transformant according to (8) by culturing the transformant;

[0011]

10 (37) A method for screening according to (34) or (36), wherein the compound that activates the G protein coupled receptor protein according to (1) is angiotensin, bombesin, canavinoïd, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, 15 purines, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedulin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, vasoactive intestinal polypeptide (VIP), somatostatin, dopamine, motilin, amylin, bradykinin, calcitonin gene-related peptide (CGRP), 20 leukotrienes, pancreastatin, prostaglandins, thromboxane, adenosine, adrenaline, α and β -chemokines (e.g., IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP-1 α , MIP-1 β , RANTES, etc.), endothelin, enterogastrin, histamin, 25 neurotensin, TRH, pancreatic polypeptide or galanin;

(38) A compound or salts thereof that alter the binding property between a ligand and the G protein coupled receptor protein or salts thereof according to (1), which is obtainable by the method for screening according to (30) through (37);

(39) A pharmaceutical composition comprising a compound or salts thereof that alter the binding property between a ligand and the G protein coupled receptor protein or salts thereof according to (1),

which is obtainable by the method for screening according to (30) through (37);

[0012]

- 5 (40) A kit for screening according to (17), comprising a cell containing the G protein coupled receptor protein according to (1);
 - 10 (41) A kit for screening according to (17), comprising a cell membrane fraction of cell containing the G protein coupled receptor protein according to (1);
 - 15 (42) A kit for screening according to (17), comprising a G protein coupled receptor protein expressed in a cell membrane of the transformant according to (8) by culturing the transformant;
 - 20 (43) A compound or salts thereof that alter the binding property between a ligand and the G protein coupled receptor protein or salts thereof according to (1), which is obtainable using the kit for screening according to (40) through (42);
 - 25 (44) A pharmaceutical composition comprising a compound or salts thereof that alter the binding property between a ligand and the G protein coupled receptor protein or salts thereof according to (1), which is obtainable using the kit for screening according to (40) through (42);
 - 30 (45) A method for quantifying the G protein coupled receptor protein according to (1), the partial peptide according to (3) or salts thereof, which comprises bringing the antibodies according to (10) in contact with the G protein coupled receptor protein according to (1), the partial peptide according to (2) or salts thereof;
 - 35 (46) A method for quantifying the G protein coupled receptor protein according to (1), the partial

peptide according to (3) or salts thereof, in a sample solution, which comprises competitively reacting the antibodies according to (10) with a sample solution and the labeled G protein coupled receptor protein
5 according to (1) or the labeled partial peptide according to (3) or salts thereof and, measuring ratio of the labeled G protein coupled receptor protein according to (1), the labeled partial peptide according to (3) or salts thereof, which are bound to the
10 antibodies; and,

(47) A method for quantifying the G protein coupled receptor protein or salts thereof according to (1) or the partial peptide or salts thereof according to (3), in a sample solution, which comprises reacting
15 a sample solution simultaneously or sequentially with antibodies of (10) immobilized on a carrier and labeled antibodies of (10) and then measuring the activity of a labeling agent on the immobilized carrier.

20 [0013]

[MODE OF EMBODIMENT OF THE INVENTION]

The G protein coupled receptor protein of the present invention (hereinafter often referred to as "receptor protein") is the receptor protein which has
25 the same or substantially the same amino acid sequence as that represented by SEQ ID NO: 1 (the amino acid sequence shown by FIGS. 1 through 3).

The receptor protein of the present invention may be any peptide derived from any cells of human and
30 another mammal (e.g., guinea pig, rat, mouse, rabbit, swine, sheep, bovine, monkey, etc.), for example, spleen cells, nerve cells, glia cells, β cells of pancreas, bone marrow cells, mesangial cells, Langerhans' cells, epidermal cells, epithelial cells,
35 endothelial cells, fibroblasts, fibrocytes, muscular

cells, fat cells, immunocytes (e.g., macrophage, T cells, B cells, natural killer cells, mast cells, neutrophils, basophils, eosinophils, monocytes, etc.), megakaryocytes, synovial cells, chondrocytes,
5 osteocytes, osteoblasts, osteoclasts, mammary gland cells, hepatocytes or interstitial cells, or precursor cells, stem cells or cancer cells of these cells and the like; cells of hemocyte type; or any tissues containing such cells, for example, brain, various
10 parts of the brain (e.g., olfactory bulb, amygdala, cerebral basal ganglia, hippocampus, thalamus, hypothalamus, substhanlamic nucleus, cerebral cortex, medulla oblongata, cerebellum, occipital pole, frontal lobe, temporal lobe, putamen, caudate nucleus, corpus
15 callosum, substantia nigra), spinal cord, pituitary, stomach, pancreas, kidney, liver, genital gland, thyroid gland, gallbladder, bone marrow, adrenal gland, skin, muscle, lung, digestive tract (e.g., large intestine, small intestine), blood vessel, heart,
20 thymus, spleen, submandibular gland, peripheral blood, peripheral hemocyte, prostate, testicle, testis, ovary, placenta, uterus, bone, joint, skeletal muscle and the like, in particular, brain and various parts of the brain. The peptide may also be a synthetic one.

25

[0014]

The amino acid sequence which has the same or substantially the same as that represented by SEQ ID NO: 1 includes an amino acid sequence having at least about 50% homology, preferably at least about 70% homology, more preferably at least about 80% homology, much more preferably at least about 90% homology, most preferably at least about 95% homology, to the amino acid sequence represented by SEQ ID NO: 1.

Specifically, the amino acid sequence includes a receptor protein comprising an amino acid sequence represented by SEQ ID NO: 5 (an amino acid sequence in FIGS. 5 to 7).

5 A preferred example of the protein having substantially the same amino acid sequence as that represented by SEQ ID NO: 1 is a protein having substantially the same amino acid sequence as that represented by SEQ ID NO: 1 and having substantially 10 the same activity as that of the amino acid sequence represented by SEQ ID NO: 1.

Examples of substantially the same activity include ligand binding activity, signal transduction activity and the like. The term "substantially the 15 same" is used to mean that the natures of their activities are equal to one another. It is thus preferred that though the activity such as ligand binding activity or signal transduction activity be equal (e.g., approximately 0.01 to 100 times, 20 preferably about 0.5 to 20 times, more preferably about 0.5 to twice), quantitative factors such as degrees of these activities and molecular weight of the proteins may differ from each other.

The activities such as ligand binding activity or 25 signal transduction activity may be determined according to a publicly known method, for example, by the method for determining ligands or the screening method which will be later described.

30 [0015]

The receptor protein of the present invention which can be used may be a protein comprising (i) an amino acid sequence represented by SEQ ID NO: 1, in which one, two, or more amino acids (preferably 1 to 30 35 amino acids, more preferably 1 to 10 amino acids, most

preferably several amino acids) are deleted; (ii) an amino acid sequence represented by SEQ ID NO: 1, to which one, two, or more amino acids (preferably 1 to 30 amino acids, more preferably 1 to 10 amino acids, most 5 preferably several amino acids) are added; (iii) an amino acid sequence represented by SEQ ID NO: 1, in which one, two, or more amino acids (preferably 1 to 30 amino acids, more preferably 1 to 10 amino acids, and most preferably several amino acids) are substituted by 10 other amino acids; and (iv) a combination of the above amino acid sequences.

The receptor protein of the present invention which can be included may be a protein comprising (i) an amino acid sequence represented by SEQ ID NO: 5, in 15 which one, two, or more amino acids (preferably 1 to 30 amino acids, more preferably 1 to 10 amino acids, most preferably several amino acids) are deleted; (ii) an amino acid sequence represented by SEQ ID NO: 5, to which one, two, or more amino acids (preferably 1 to 30 20 amino acids, more preferably 1 to 10 amino acids, most preferably several amino acids) are added; (iii) an amino acid sequence represented by SEQ ID NO: 5, in which one, two, or more amino acids (preferably 1 to 30 amino acids, more preferably 1 to 10 amino acids, and 25 most preferably several amino acids) are substituted by other amino acids; and (iv) a combination of the above amino acid sequences.

[0016]

30 The receptor protein of the present invention is designated by the art-recognized way of describing peptides. That is, the left end (amino terminal) is the N-terminal and the right end (carboxyl terminal) is the C-terminal. In the receptor protein of the present 35 invention comprising an amino acid sequence represented

by SEQ ID No: 1, the C-terminal is normally carboxyl group (-COOH) or carboxylate (-COO⁻), but the C-terminal may be an amide (-CONH₂) or an ester (-COOR).

Examples of the ester group shown by R include an alkyl group having 1 to 6 carbon atoms such as methyl, ethyl, n-propyl, isopropyl, n-butyl, etc.; a cycloalkyl group having 3 to 8 carbon atoms such as cyclopentyl, cyclohexyl, etc.; an aryl group having 6 to 12 carbon atoms such as phenyl, α-naphthyl, etc.; an aralkyl having 7 to 14 carbon atoms such as a phenyl-C₁₋₂ alkyl group, e.g., benzyl, phenethyl, etc.; an α-naphthyl-C₁₋₂ alkyl group, e.g., α-naphthylmethyl, etc.; and the like. In addition, pivaloyloxymethyl or the like which is used widely as an ester for oral administration may also be used.

Where the receptor protein of the present invention contains a carboxyl group (or carboxylate) at a position other than the C-terminal, it may be amidated or esterified and such an amide or ester is also included within the receptor protein of the present invention. The ester group may be the same group as that described with respect to the above C-terminal.

Further, the receptor protein of the present invention includes derivatives wherein the amino group of N-terminal methionine residue of the above protein is protected with a protecting group (e.g., an acyl group having 1 to 6 carbon atoms such as formyl group, acetyl group, etc.); those wherein the N-terminal region is cleaved in vivo and the glutamyl group formed is pyroglutaminated; and those wherein a substituent (e.g., -OH, -COOH, amino group, imidazole group, indole group, guanidino group, etc.) on the side chains of an amino acid in the molecule of the protein is protected with an appropriate protecting group (e.g., an acyl

group having 1 to 6 carbon atoms such as an alkanoyl group having 2 to 6 carbon atoms, e.g., formyl group, acetyl group, etc.), or conjugated proteins such as glycoproteins with sugar chains attached thereto.

5 Specific examples of the receptor protein of the present invention which can be used include a rat-derived receptor protein containing the amino acid sequence represented by SEQ ID NO: 1 or a human-derived receptor protein containing the amino acid sequence
10 represented by SEQ ID NO: 5.

[0017].

The partial peptide of the receptor protein of the present invention (hereinafter sometimes referred to as
15 the partial peptide) may be any partial peptide, so long as it constitutes a part of the peptide portions of the receptor protein of the present invention described above. Examples of such partial peptide include the site, which is exposed outside cell
20 membranes among the receptor protein molecule of the present invention and retains the receptor binding activity.

An example of the partial peptide of the protein containing the amino acid sequence represented by SEQ
25 ID NO: 1 is a peptide containing a region which is analyzed to be an extracellular area (hydrophilic region or site) in a hydrophobic plotting analysis shown by FIG. 4. Further, an example of the partial peptide of the protein containing the amino acid
30 sequence represented by SEQ ID NO: 5 is a peptide containing a region which is analyzed to be an extracellular area (hydrophilic region or site) in a hydrophobic plotting analysis shown by FIG. 8. A peptide, which partly contains a hydrophobic region or
35 site, may be used as well. Further, a peptide, which

independently contains each domain, may also be used although the partial peptide, which contains a plurality of domains at the same time, may also be used.

The number of amino acids in the partial peptide 5 of the present invention is at least 20 or more, preferably 50 or more, more preferably 100 or more, in terms of the constructive amino acid sequence of the receptor protein of the present invention described above.

10 The substantially the same amino acid sequence refers to an amino acid sequence having at least about 50% homology, preferably at least about 70% homology, more preferably at least about 80% homology, much more preferably at least about 90% homology, most preferably 15 at least about 95% homology.

Herein, the term "substantially the same activity" has the same definition described above. The "substantially the same activity" can be assayed in the manner described above.

20

[0018]

In the partial peptide of the present invention, one, two, or more amino acids (preferably approximately 1 to 10 amino acids, more preferably several amino acids) may be deleted in the amino acid sequence 25 described above; one, two, or more amino acids (preferably approximately 1 to 20 amino acids, more preferably approximately 1 to 10 amino acids, and most preferably several amino acids) may be added to the amino acid sequence; or, one, two, or more amino acids 30 (preferably approximately 1 to 10 amino acids, more preferably several amino acids, most preferably approximately 1 to 5 amino acids) may be substituted by other amino acids in the amino acid sequence.

In the partial peptide of the present invention, the C-terminal is normally carboxyl group (-COOH) or carboxylate (-COO⁻) but the C-terminal may be an amide (-CONH₂) or an ester (-COOR), as has been described
5 with the receptor protein of the present invention.

As in the receptor protein of the present invention described above, the partial peptide of the present invention also includes conjugated peptides such as those in which the amino group of the N-terminal methionine residue is protected by a
10 protecting group, those in which the N-terminal residue is cleaved in vivo and the produced Gln is converted into pyroglutamate, those in which substituents on the side chains of amino acids in the molecule are
15 protected by appropriate protecting groups and those to which sugar chains are bound, that is, glycopeptides.

In the partial peptide of the present invention, the C-terminal is normally carboxyl group (-COOH) or carboxylate (-COO⁻) but the C-terminal may be an amide
20 (-CONH₂) or an ester (-COOR), as has been described with the receptor protein of the present invention.

The receptor protein of the present invention or partial peptide thereof may be used preferably in the form of physiologically acceptable acid addition salts
25 thereof. Examples of such salts are salts with inorganic acids (e.g., hydrochloric acid, phosphoric acid, hydrobromic acid, sulfuric acid), salts with organic acids (e.g., acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid, benzenesulfonic acid) and the like.

The receptor protein of the present invention or salts thereof may be manufactured in accordance with a publicly known method for purification of a receptor protein from human or other mammalian cells or tissues 5 described above. Alternatively, the receptor protein of the present invention or salts thereof may also be manufactured by culturing a transformant containing DNA encoding the receptor protein of the present invention, as will be later described. Furthermore the receptor 10 protein of the present invention or salts thereof may also be manufactured by the methods for synthesizing proteins, which will also be described hereinafter, or by modified methods.

Where the receptor protein or salts thereof are 15 manufactured from human or mammalian tissues or cells, human or mammalian tissues or cells are homogenized, then extracted with an acid or the like, and the extract is isolated and purified by a combination of chromatography techniques such as reverse phase 20 chromatography, ion exchange chromatography, and the like.

[0020]

To synthesize the receptor protein, partial peptide or its salts or amides of the present invention, commercially available resins that are used for protein synthesis can be used. Examples of such resins include chloromethyl resin, hydroxymethyl resin, benzhydrylamine resin, aminomethyl resin, 4- 25 benzylxybenzyl alcohol resin, 4-methylbenzhydrylamine resin, PAM resin, 4-hydroxymethylmethyphenyl acetamidomethyl resin, polyacrylamide resin, 4-(2',4'-dimethoxyphenyl-hydroxymethyl)phenoxy resin and 4-(2',4'-dimethoxyphenyl-Fmoc-aminoethyl) phenoxy resin. 30 Using these resins, amino acids in which α-amino groups 35

and functional groups on the side chains are appropriately protected are condensed on the resin in the order of the sequence of the objective protein according to various condensation methods publicly
5 known in the art. At the end of the reaction, the protein is excised from the resin and at the same time, the protecting groups are removed. Then, intramolecular disulfide bond-forming reaction is performed in a highly diluted solution to obtain the
10 objective protein or amides thereof.

For condensation of the protected amino acids described above, a variety of activation reagents for protein synthesis may be used, but carbodiimides are particularly preferably employed. Examples of such
15 carbodiimides include DCC, N,N'-diisopropylcarbodiimide, and N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide. For activation by these reagents, the protected amino acids in combination with a racemization inhibitor (e.g., HOEt, HOOEt) are added directly to the resin, or
20 the protected amino acids are previously activated in the form of symmetric acid anhydrides, HOEt esters or HOOEt esters, followed by adding the thus activated protected amino acids to the resin.

25 [0021]

Solvents suitable for use in the activation of the protected amino acids or the condensation with the resin may be selected from solvents that are known to be usable for protein condensation reactions. Examples
30 of such solvents are acid amides such as N,N-dimethylformamide, N,N-dimethylacetamide and N-methylpyrrolidone; halogenated hydrocarbons such as methylene hydrochloride and chloroform; alcohols such as trifluoroethanol; sulfoxides such as
35 dimethylsulfoxide; ethers such as pyridine, dioxane and

tetrahydrofuran; nitriles such as acetonitrile and propionitrile; esters such as methyl acetate and ethyl acetate; and appropriate mixtures of these solvents. The reaction temperature is appropriately chosen from 5 the range known to be applicable to protein bonding reactions and is usually selected in the range of approximately -20°C to 50°C. The activated amino acid derivatives are used generally in an excess of 1.5 to 4 times. The condensation is examined using the 10 ninhydrin reaction; when the condensation is insufficient, the condensation can be completed by repeating the condensation reaction without removal of the protecting groups. When the condensation is yet insufficient even after repeating the reaction, 15 unreacted amino acids are acetylated with acetic anhydride or acetylimidazole.

[0022]

Examples of the protecting groups used to protect 20 the starting amino groups include Z, Boc, t-pentyloxycarbonyl, isobornyloxycarbonyl, 4-methoxybenzyloxycarbonyl, Cl-Z, Br-Z, adamantlyloxycarbonyl, trifluoroacetyl, phthaloyl, formyl, 2-nitrophenylsulphenyl, diphenylphosphinothioyl, 25 and Fmoc.

A carboxyl group can be protected by, e.g., alkyl esterification (in the form of linear, branched or cyclic alkyl esters of the alkyl moiety such as methyl, ethyl, propyl, butyl, t-butyl, cyclopentyl, cyclohexyl, 30 cycloheptyl, cyclooctyl and 2-adamantyl), aralkyl esterification (e.g., benzyl ester, 4-nitrobenzyl ester, 4-methoxybenzyl ester, 4-chlorobenzyl ester and benzhydryl ester), phenacyl esterification, benzyloxycarbonyl hydrazidation, t-butoxycarbonyl 35 hydrazidation and trityl hydrazidation.

The hydroxyl group of serine can be protected through, for example, its esterification or etherification. Examples of groups appropriately used for the esterification include a lower alkanoyl group such as acetyl group, an aroyl group such as benzoyl group, and a group derived from carbonic acid such as benzyloxycarbonyl group and ethoxycarbonyl group. Examples of a group appropriately used for the etherification include benzyl group, tetrahydropyranyl group and t-butyl group.

10 Examples of groups for protecting the phenolic hydroxyl group of tyrosine include Bzl, Cl₂-Bzl, 2-nitrobenzyl, Br-Z and t-butyl.

15 Examples of groups used to protect the imidazole moiety of histidine include Tos, 4-methoxy-2,3,6-trimethylbenzenesulfonyl, DNP, benzyloxymethyl, Bum, Boc, Trt and Fmoc.

[0023]

20 Examples of the activated carboxyl groups in the starting amino acids include the corresponding acid anhydrides, azides, activated esters (esters with alcohols (e.g., pentachlorophenol, 2,4,5-trichlorophenol, 2,4-dinitrophenol, cyanomethyl alcohol, 25 p-nitrophenol, HONB, N-hydroxysuccimide, N-hydroxyphthalimide, HOEt). As the activated amino acids in which the amino groups are activated in the starting material, the corresponding phosphoric amides are employed.

30 To eliminate (split off) the protecting groups, there are used catalytic reduction under hydrogen gas flow in the presence of a catalyst such as Pd-black or Pd-carbon; an acid treatment with anhydrous hydrogen fluoride, methanesulfonic acid, trifluoromethanesulfonic acid or trifluoroacetic acid,

or a mixture solution of these acids; a treatment with a base such as diisopropylethylamine, triethylamine, piperidine or piperazine; and reduction with sodium in liquid ammonia. The elimination reaction of the 5 protecting group by the acid treatment described above is carried out generally at a temperature of approximately -20°C to 40°C. In the acid treatment, it is efficient to add a cation scavenger such as anisole, phenol, thioanisole, m-cresol, p-cresol, 10 dimethylsulfide, 1,4-butanedithiol or 1,2-ethanedithiol. Furthermore, 2,4-dinitrophenyl group known as the protecting group for the imidazole of histidine is removed by a treatment with thiophenol. Formyl group used as the protecting group of the indole of 15 tryptophan is eliminated by the aforesaid acid treatment in the presence of 1,2-ethanedithiol or 1,4-butanedithiol, as well as by a treatment with an alkali such as a dilute sodium hydroxide solution and dilute ammonia.

20

[0024]

Protection of functional groups that should not be involved in the reaction of the starting materials, 25 protecting groups, elimination of the protecting groups and activation of functional groups involved in the reaction may be appropriately selected from publicly known groups and publicly known means.

In another method for obtaining amidated proteins, for example, the α -carboxyl group of the carboxy 30 terminal amino acid is first protected by amidation; the peptide (protein) chain is then extended from the amino group side to a desired length. Thereafter, a protein in which only the protecting group of the N-terminal α -amino group has been eliminated from the 35 peptide and a protein in which only the protecting

group of the C-terminal carboxyl group has been
eliminated are manufactured. The two proteins are
condensed in a mixture of the solvents described above.
The details of the condensation reaction are the same
5 as described above. After the protected protein
obtained by the condensation is purified, all the
protecting groups are eliminated by the method
described above to give the desired crude protein.
This crude protein is purified by various known
10 purification means. Lyophilization of the major
fraction gives the amide of the desired protein.

To prepare the esterified protein, for example,
the α -carboxyl group of the carboxy terminal amino
acid is condensed with a desired alcohol to prepare the
15 amino acid ester, which is followed by procedure
similar to the preparation of the amidated protein
above to give the desired esterified protein.

[0025]

20 The partial peptide of the receptor protein of the
present invention can be manufactured by publicly known
methods for peptide synthesis, or by cleaving the
receptor protein of the present invention with an
appropriate peptidase. For the methods for peptide
25 synthesis, for example, either solid phase synthesis or
liquid phase synthesis may be used. That is, the
partial peptide or amino acids that can construct the
receptor protein of the present invention are condensed
with the remaining part of the receptor protein of the
30 present invention. Where the product contains
protecting groups, these protecting groups are removed
to give the desired peptide. Publicly known methods
for condensation and elimination of the protecting
groups are described in 1) - 5) below.

- 1) M. Bodanszky & M.A. Ondetti: *Peptide Synthesis*, Interscience Publishers, New York (1966)
 - 2) Schroeder & Luebke: *The Peptide*, Academic Press, New York (1965)
 - 5 3) Nobuo Izumiya, et al.: *Peptide Gosei-no-Kiso to Jikken* (Basics and experiments of peptide synthesis), published by Maruzen Co. (1975)
 - 4) Haruaki Yajima & Shunpei Sakakibara: *Seikagaku Jikken Koza* (Biochemical Experiment) 1, *Tanpakushitsu no Kagaku* (Chemistry of Proteins) IV, 205 (1977)
 - 10 5) Haruaki Yajima ed.: *Zoku Iyakuhin no Kaihatsu* (A sequel to Development of Pharmaceuticals), Vol. 14, Peptide Synthesis, published by Hirokawa Shoten
- 15 After completion of the reaction, the product may be purified and isolated by a combination of conventional purification methods such as solvent extraction, distillation, column chromatography, liquid chromatography and recrystallization to give the
- 20 partial peptide of the present invention. When the partial peptide obtained by the above methods is in a free form, the peptide can be converted into an appropriate salt by a publicly known method; when the protein is obtained in a salt form, it can be converted
- 25 into a free form by a publicly known method.

[0026]

- The polynucleotide encoding the receptor protein of the present invention may be any polynucleotide so long as it contains the base sequence (DNA or RNA, preferably DNA) encoding the receptor protein of the present invention described above. Such a polynucleotide may be DNA and RNA including mRNA that codes for the receptor protein of the present invention.
- 35 The polynucleotide may be either double-stranded or

single-stranded. When the polynucleotide is double-stranded, it may be double-stranded DNA or a DNA:RNA hybrid. When the polynucleotide is single-stranded, it may be a sense strand (i.e., coding strand) or an
5 antisense strand (i.e., non-coding strand).

Using the polynucleotide encoding the receptor protein of the present invention, mRNA of the receptor protein of the present invention can be quantified by, for example, the method published in separate volume of
10 *Jikken Igaku* (Experimental Medical Science), 15(7), "New PCR and its application" (1997) or by modification of the method.

The DNA encoding the receptor protein of the present invention may be any of genomic DNA, genomic
15 DNA library, cDNA derived from the cells and tissues described above, cDNA library derived from the cells and tissues described above and synthetic DNA. The vector to be used for the library may be any of bacteriophage, plasmid, cosmid and phagemid. The DNA
20 may be directly amplified by reverse transcriptase polymerase chain reaction (hereinafter abbreviated as RT-RCR) using the total RNA or mRNA fraction prepared from the cells and tissues described above.

Specifically, the DNA encoding the partial peptide
25 of the receptor protein of the present invention may be any DNA so long as it has, for example, DNA having the amino acid sequence represented by SEQ ID NO: 2 or SEQ ID NO: 6, or it has a base sequence hybridizable to the base sequence represented by SEQ ID NO: 2 or SEQ ID NO:
30 6 under high stringent conditions and encodes a receptor protein having substantially the same activities (i.e., ligand binding activity, signal transduction activity and the like) as those of the receptor protein of the present invention.

Examples of the DNA that is hybridizable to the base sequence represented by SEQ ID NO: 2 or SEQ ID NO: 6 include DNA having at least about 70% homology, preferably at least about 80% homology, more preferably 5 at least about 90% homology, most preferably at least about 95% homology, to the amino acid sequence represented by SEQ ID NO: 2 or SEQ ID NO: 6.

[0027]

10 The hybridization can be carried out by publicly known methods or by a modification thereof, for example, according to the method described in Molecular Cloning, 2nd Ed.; J. Sambrook et al., Cold Spring Harbor Lab. Press, (1989). A commercially available library may 15 also be used according to the instructions of the attached manufacturer's protocol. The hybridization can be carried out preferably under high stringent conditions.

20 The high stringent conditions used herein are, for example, those in a sodium concentration at about 19 mM to about 40 mM, preferably about 19 mM to about 20 mM and a temperature at about 50°C to about 70°C, preferably about 60°C to about 65°C. In particular, 25 hybridization conditions in a sodium concentration at about 19 mM and a temperature at about 65°C are most preferred.

More specifically, for the DNA encoding the receptor protein having the amino acid sequence represented by SEQ ID NO: 1, there may be employed DNA 30 having the base sequence represented by SEQ ID NO: 2. Further, for the DNA encoding the receptor protein having the amino acid sequence represented by SEQ ID NO: 5, there may be employed DNA having the base sequence represented by SEQ ID NO: 6.

The polynucleotide comprising a part of the base sequence of DNA encoding the receptor protein of the present invention or a part of the base sequence complementary to the DNA is intended to include not 5 only DNA encoding the partial peptide of the present invention described below but also RNA.

According to the present invention, antisense polynucleotides (nucleic acids) that can inhibit replication or expression of the G protein coupled 10 receptor protein gene can be designed and synthesized based on the cloned or determined base sequence information of the DNA encoding the G protein coupled receptor protein. Such polynucleotides (nucleic acids) can hybridize to the RNA of the G protein coupled 15 receptor protein gene and inhibit RNA synthesis or the function of RNA, or can regulate/control the expression of the G protein coupled receptor protein gene via the interaction with RNAs associated with the G protein coupled receptor protein. Polynucleotides 20 complementary to the specified sequences of RNA associated with the G protein coupled receptor protein and polynucleotides that can specifically hybridize to RNA associated with the G protein coupled receptor protein are useful for regulating and controlling the 25 expression of the G protein coupled receptor protein gene in vivo and in vitro. These polynucleotides are also useful for the treatment and diagnosis of diseases. The term "correspond" is used to refer to homologous or 30 complementary to a specific sequence of nucleotides, base sequences or nucleic acids including the gene. As between nucleotides, base sequences or nucleic acids and peptides (proteins), the term "corresponding" usually refers to amino acids of a peptide (protein) 35 that is instructed to be derived from the sequence of nucleotides (nucleic acids) or its complements. The 5'

end hairpin loop, 5' end 6-base-pair repeats, 5' end untranslated region, polypeptide translation initiation codon, protein coding region, ORF translation initiation codon, 3' untranslated region, 3' end 5 palindrome region, and 3' end hairpin loop of the G protein coupled receptor protein gene may be selected as preferred target regions, though any region may be a target within the G protein coupled receptor protein genes.

10

[0028]

The relationship between the targeted nucleic acids and the polynucleotides complementary to at least a portion of the target, specifically the relationship 15 between the target and the polynucleotides hybridizable to the target, is denoted to be "antisense". The antisense polynucleotides may be polydeoxynucleotides containing 2-deoxy-D-ribose, polydeoxynucleotides containing D-ribose, any other type of polynucleotides 20 which are N-glycosides of a purine or pyrimidine base, or other polymers containing non-nucleotide backbones (e.g., protein nucleic acids and synthetic sequence-specific nucleic acid polymers commercially available) or other polymers containing nonstandard linkages 25 (provided that the polymers contain nucleotides with such a configuration that allows base pairing or base stacking, as is found in DNA and RNA). The antisense polynucleotides may be double-stranded DNA, single-stranded DNA, double-stranded RNA, single-stranded RNA 30 or a DNA:RNA hybrid, and further includes unmodified polynucleotides (or unmodified oligonucleotides), those with publicly known types of modifications, for example, those with labels known in the art, those with caps, methylated polynucleotides, those with substitution of 35 one or more of naturally occurring nucleotides with

their analogue, those with intramolecular modifications of nucleotides such as those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, etc.) and those with charged linkages or sulfur-containing linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those having side chain groups such as proteins (including nucleases, nuclease inhibitors, toxins, antibodies, signal peptides, poly-L-lysine, etc.) and saccharides (e.g., monosaccharides, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.) and those containing alkylating agents, those with modified linkages (e.g., α anomeric nucleic acids, etc.). Herein the terms "nucleoside", "nucleotide" and "nucleic acid" are used to refer to moieties that contain not only the purine and pyrimidine bases, but also other heterocyclic bases, which have been modified. Such modifications include methylated purines and pyrimidines, acylated purines and pyrimidines and other heterocyclic rings. Modified nucleosides or nucleotides also include modifications on the sugar moiety, for example, wherein one or more hydroxyl groups may optionally be replaced with a halogen, aliphatic groups, or may be converted into the corresponding functional groups such as ethers, amines, or the like.

[0029]

The antisense polynucleotide (nucleic acid) of the present invention is RNA, DNA or a modified nucleic acid (RNA, DNA). Specific examples of the modified nucleic acid are, but not limited to, sulfurized and thiophosphate derivatives of nucleic acids and those resistant to degradation of polynucleoside or

oligonucleoside amides. The antisense nucleic acids of the present invention can be modified preferably based on the following design, that is, by increasing the intracellular stability of the antisense nucleic acid,
5 increasing the cellular permeability of the antisense nucleic acid, increasing the affinity of the nucleic acid to the target sense strand to a higher level, or minimizing the toxicity, if any, of the antisense nucleic acid.

10 Many such modifications are known in the art, as disclosed in J. Kawakami et al., Pharm. Tech. Japan, Vol. 8, pp.247, 1992; Vol. 8, pp.395, 1992; S. T. Crooke et al. ed., Antisense Research and Applications, CRC Press, 1993; etc.

15 The antisense nucleic acid of the present invention may contain altered or modified sugars, bases or linkages. The antisense nucleic acid may also be provided in a specialized form such as liposomes, microspheres or may be applied to gene therapy or may
20 be provided in combination with attached moieties. Such attached moieties include polycations such as polylysine that act as charge neutralizers of the phosphate backbone, or hydrophobic moieties such as lipids (e.g., phospholipids, cholesterol, etc.) that
25 enhance the interaction with cell membranes or increase uptake of the nucleic acid. Preferred examples of the lipids to be attached are cholesterol or derivatives thereof (e.g., cholesteryl chloroformate, cholic acid, etc.). These moieties may be attached at the 3' or 5'
30 ends of the nucleic acid and may be also attached through a base, sugar, or intramolecular nucleoside linkage. Other moieties may be capping groups specifically placed at the 3' or 5' ends of the nucleic acid to prevent degradation by nuclease such as
35 exonuclease, RNase, etc. Such capping groups include,

but are not limited to, hydroxyl protecting groups known in the art, including glycols such as polyethylene glycol, tetraethylene glycol and the like.

The inhibitory activity of the antisense nucleic acid can be examined using the transformant of the present invention, the gene expression system of the present invention in vitro and in vivo, or the translation system of the G protein coupled receptor protein of the present invention in vitro and in vivo.
10 The nucleic acid can be applied to cells by a variety of publicly known methods.

[0030]

The DNA encoding the partial peptide of the present invention may be any DNA so long as it contains the base sequence encoding the partial peptide of the present invention described above. The DNA may also be any of genomic DNA, genomic DNA library, cDNA derived from the cells and tissues described above, cDNA library derived from the cells and tissues described above and synthetic DNA. The vector to be used for the library may be any of bacteriophage, plasmid, cosmid and phagemid. The DNA may be directly amplified by reverse transcriptase polymerase chain reaction
25 (hereinafter simply referred to as RT-RCR) using mRNA fraction prepared from the cells and tissues described above.

Specific examples of the DNA encoding the partial peptide of the present invention include (1) DNA that has a part of the base sequence of the DNA containing the base sequence represented by SEQ ID NO: 2 or SEQ ID NO: 6 and (2) DNA having a base sequence hybridizable to the base sequence represented by SEQ ID NO: 2 or SEQ ID NO: 6 under high stringent conditions and containing
35 a part of the base sequence of the DNA encoding a

receptor protein having substantially the same activities (i.e., ligand binding activity, signal transduction activity and the like) as those of the receptor protein peptide of the present invention.

5 Examples of the DNA that is hybridizable to the base sequence represented by SEQ ID NO: 2 or SEQ ID NO: 6 include DNA containing the base sequence having at least about 70% homology, preferably at least about 80% homology, more preferably at least about 90% homology, 10 most preferably at least about 95% homology, to the base sequence represented by SEQ ID NO: 2 or SEQ ID NO: 6.

[0031]

15 For cloning of the DNA that completely encodes the receptor protein or its partial peptide of the present invention (hereinafter sometimes referred to as the receptor protein of the present invention), the DNA may be either amplified by PCR using synthetic DNA primers 20 containing a part of the base sequence of the receptor protein of the present invention, or the DNA inserted into an appropriate vector can be selected by hybridization with a labeled DNA fragment or synthetic DNA that encodes a part or entire region of the 25 receptor protein of the present invention. The hybridization can be carried out, for example, according to the method described in Molecular Cloning, 2nd (J. Sambrook et al., Cold Spring Harbor Lab. Press, 1989). The hybridization may also be performed using 30 commercially available library in accordance with the protocol described in the attached instructions.

[0032]

Conversion of the base sequence of DNA can be 35 carried out according to publicly known methods such as

the Guppy duplex method or the Kunkel method or its modification by using a publicly known kit available as MutanTM-G or MutanTM-K (both manufactured by Takara Shuzo Co., Ltd., trademark).

5 The cloned DNA encoding the receptor protein can be used as it is, depending upon purpose or, if desired, after digestion with a restriction enzyme or after addition of a linker thereto. The DNA may contain ATG as a translation initiation codon at the 5' end thereof and TAA, TGA or TAG as a translation termination codon at the 3' end thereof. These translation initiation and termination codons may also be added by using an appropriate synthetic DNA adapter.

10

15 The expression vector of the receptor protein of the present invention can be manufactured, for example, by (a) excising the desired DNA fragment from the DNA encoding the receptor protein of the present invention, (b) followed by ligation of the DNA fragment with an appropriate expression vector downstream a promoter in
20 the vector.

[0033]

Examples of the vector which can be used include plasmids derived form E. coli (e.g., pBR322, pBR325, 25 pUC12, pUC13), plasmids derived from Bacillus subtilis (e.g., pUB110, pTP5, pC194), plasmids derived from yeast (e.g., pSH19, pSH15), bacteriophages such as λ phage, etc., animal viruses such as retrovirus, vaccinia virus, baculovirus, etc. as well as pA1-11, 30 pXT1, pRc/CMV, pRc/RSV, pcDNAI/Neo, etc.

The promoter used in the present invention may be any promoter if it matches well with a host to be used for gene expression. In the case of using animal cells as the host, examples of the promoter include SRα 35 promoter, SV40 promoter, LTR promoter, CMV promoter,

HSV-TK promoter, etc. Among them, CMV promoter or SR α promoter is preferably used.

Where the host is bacteria of the genus Escherichia, preferred examples of the promoter include 5 trp promoter, lac promoter, recA promoter, λ PL promoter, lpp promoter, etc. In the case of using bacteria of the genus Bacillus as the host, preferred example of the promoter are SPO1 promoter, SPO2 promoter and penP promoter. In the case of using yeast as the host, 10 preferred examples of the promoter are PHO5 promoter, PGK promoter, GAP promoter and ADH promoter. In the case of using insect cells as the host, preferred examples of the promoter include polyhedrin prompter and P10 promoter.

15

[0034]

In addition to the foregoing examples, the expression vector may further optionally contain an enhancer, a splicing signal, a poly A addition signal, 20 a selection marker, SV40 replication origin (hereinafter sometimes abbreviated as SV40ori) etc. Examples of the selection marker include dihydrofolate reductase (hereinafter sometimes abbreviated as dhfr) gene [methotrexate (MTX) resistance], ampicillin 25 resistant gene (hereinafter sometimes abbreviated as Amp^r), neomycin resistant gene (hereinafter sometimes abbreviated as Neo, G418 resistance), etc. In particular, when CHO (dhfr^r) cell is used together with dhfr gene as the selection marker, selection can also 30 be made by using a thymidine free medium.

If necessary and desired, a signal sequence that matches with a host is added to the N-terminus of the receptor protein of the present invention. Examples of the signal sequence that can be used are Pho A signal 35 sequence, OmpA signal sequence, etc. in the case of

using bacteria of the genus Escherichia as the host; α -amylase signal sequence, subtilisin signal sequence, etc. in the case of using bacteria of the genus Bacillus as the host; MFA signal sequence, SUC2 signal sequence, etc. in the case of using yeast as the host; and insulin signal sequence, α -interferon signal sequence, antibody molecule signal sequence, etc. in the case of using animal cells as the host, respectively.

10 Using the vector comprising the DNA encoding the receptor protein of the present invention thus constructed, transformants can be manufactured.

[0035]

15 Examples of the host, which may be employed, are bacteria belonging to the genus Escherichia, bacteria belonging to the genus Bacillus, yeast, insect cells, insects and animal cells, etc.

20 Specific examples of bacteria belonging to the genus Escherichia include Escherichia coli K12 DH1 (Proc. Natl. Acad. Sci. U.S.A., 60, 160 (1968)), JM103 (Nucleic Acids Research, 9, 309 (1981)), JA221 (Journal of Molecular Biology, 120, 517 (1978)), HB101 (Journal of Molecular Biology, 41, 459 (1969)), C600 (Genetics, 39, 440 (1954)), etc.

25 Examples of bacteria belonging to the genus Bacillus include Bacillus subtilis MI114 (Gene, 24, 255 (1983)), 207-21 (Journal of Biochemistry, 95, 87 (1984)), etc.

30 Examples of yeast include Saccharomyces cereviseae AH22, AH22R, NA87-11A, DKD-5D, 20B-12, Schizosaccharomyces pombe NCYC1913, NCYC2036, Pichia pastoris, etc.

Examples of insect cells include, for the virus AcNPV, Spodoptera frugiperda cell (Sf cell), MG1 cell derived from mid-intestine of Trichoplusia ni, High Five™ cell derived from egg of Trichoplusia ni, cells 5 derived from Mamestra brassicae, cells derived from Estigmena acrea, etc.; and for the virus BmNPV, Bombyx mori N cell (BmN cell), etc. Examples of the Sf cell which can be used are Sf9 cell (ATCC CRL1711) and Sf21 cell (both cells are described in Vaughn, J. L. et al.,
10 In Vivo, 13, 213-217 (1977).

As the insect, for example, a larva of Bombyx mori can be used (Maeda et al., Nature, 315, 592 (1985)).

Examples of animal cells include monkey cell COS-7, Vero, Chinese hamster cell CHO (hereinafter referred to 15 as CHO cell), dhfr gene deficient Chinese hamster cell CHO (hereinafter simply referred to as CHO(dhfr') cell), mouse L cell, mouse AtT-20, mouse myeloma cell, rat GH 3, human FL cell, etc.

20 [0037]

Bacteria belonging to the genus Escherichia can be transformed, for example, according to the method described in Proc. Natl. Acad. Sci. U.S.A., 69, 2110 (1972) or Gene, 17, 107 (1982).

25 Bacteria belonging to the genus Bacillus can be transformed, for example, according to the method described in Molecular & General Genetics, 168, 111 (1979).

Yeast can be transformed, for example, according 30 to the method described in Methods in Enzymology, 194, 182-187 (1991) or Proc. Natl. Acad. Sci. U.S.A., 75, 1929 (1978).

Insect cells or insects can be transformed, for example, according to the method described in
35 Bio/Technology, 6, 47-55(1988).

Animal cells can be transformed, for example, according to the method described in *Saibo Kogaku* (Cell Engineering), extra issue 8, *Shin Saibo Kogaku Jikken Protocol* (New Cell Engineering Experimental Protocol), 5 263-267 (1995), published by Shujunsha, or *Virology*, 52, 456 (1973).

Thus, the transformant transformed with the expression vector containing the DNA encoding the G protein coupled receptor protein can be obtained.

10 Where the host is bacteria belonging to the genus Escherichia or the genus Bacillus, the transformant can be appropriately incubated in a liquid medium which contains materials required for growth of the transformant such as carbon sources, nitrogen sources, 15 inorganic materials, etc. Examples of the carbon sources include glucose, dextrin, soluble starch, sucrose, etc. Examples of the nitrogen sources include inorganic or organic materials such as ammonium salts, nitrate salts, corn steep liquor, peptone, casein, meat 20 extract, soybean cake, potato extract, etc. Examples of the inorganic materials are calcium chloride, sodium dihydrogenphosphate, magnesium chloride, etc. In addition, yeast, vitamins, growth promoting factors etc. may also be added to the medium. Preferably, pH of the 25 medium is adjusted to about 5 to about 8.

[0038]

A preferred example of the medium for incubation of the bacteria belonging to the genus Escherichia is 30 M9 medium supplemented with glucose and Casamino acids (Miller, *Journal of Experiments in Molecular Genetics*, 431-433, Cold Spring Harbor Laboratory, New York, 1972). If necessary and desired, a chemical such as 3 β -indolylacrylic acid can be added to the medium thereby 35 to activate the promoter efficiently. Where the

bacteria belonging to the genus Escherichia are used as the host, the transformant is usually cultivated at about 15°C to about 43°C for about 3 hours to about 24 hours. If necessary and desired, the culture may be 5 aerated or agitated.

Where the bacteria belonging to the genus Bacillus are used as the host, the transformant is cultivated generally at about 30°C to about 40°C for about 6 hours to about 24 hours. If necessary and desired, the 10 culture can be aerated or agitated.

Where yeast is used as the host, the transformant is cultivated in, for example, Burkholder's minimal medium (Bostian, K. L. et al., Proc. Natl. Acad. Sci. U.S.A., 77, 4505 (1980)) or in SD medium supplemented 15 with 0.5% Casamino acids (Bitter, G. A. et al., Proc. Natl. Acad. Sci. U.S.A., 81, 5330 (1984)). Preferably, pH of the medium is adjusted to about 5 to about 8. In general, the transformant is cultivated at about 20°C to about 35°C for about 24 hours to about 72 hours. If 20 necessary and desired, the culture can be aerated or agitated.

[0039]

Where insect cells or insects are used as the host, 25 the transformant is cultivated in, for example, Grace's Insect Medium (Grace, T. C. C., Nature, 195, 788 (1962)) to which an appropriate additive such as immobilized 10% bovine serum is added. Preferably, pH of the medium is adjusted to about 6.2 to about 6.4. 30 Normally, the transformant is cultivated at about 27°C for about 3 days to about 5 days and, if necessary and desired, the culture can be aerated or agitated.

Where animal cells are employed as the host, the transformant is cultivated in, for example, MEM medium 35 containing about 5% to about 20% fetal bovine serum

(Science, 122, 501 (1952)), DMEM medium (Virology, 8, 396 (1959)), RPMI 1640 medium (The Journal of the American Medical Association, 199, 519 (1967)), 199 medium (Proceeding of the Society for the Biological Medicine, 73, 1 (1950)), etc. Preferably, pH of the medium is adjusted to about 6 to about 8. The transformant is usually cultivated at about 30°C to about 40°C for about 15 hours to about 60 hours and, if necessary and desired, the culture can be aerated or agitated.

As described above, the G protein coupled receptor protein of the present invention can be produced in the cell membrane of the transformant.

15 [0040]

The receptor protein of the present invention can be separated and purified from the culture described above by the following procedures.

When the receptor protein of the present invention is extracted from the culture or cells, after cultivation, the transformant or cell is collected by a publicly known method and suspended in a appropriate buffer. The transformant or cell is then disrupted by publicly known methods such as ultrasonication, a treatment with lysozyme and/or freeze-thaw cycling, followed by centrifugation, filtration, etc. Thus, the crude extract of the receptor protein can be obtained. The buffer used for the procedures may contain a protein modifier such as urea or guanidine hydrochloride, or a surfactant such as Triton X-100TM, etc. When the receptor protein of the present invention is secreted in the culture broth, after completion of the cultivation the supernatant can be separated from the transformant or cell to collect the supernatant by a publicly known method.

The supernatant or the receptor protein contained in the extract thus obtained can be purified by appropriately combining the publicly known methods for separation and purification. Such publicly known 5 methods for separation and purification include a method utilizing difference in solubility such as salting out, solvent precipitation, etc.; a method mainly utilizing difference in molecular weight such as dialysis, ultrafiltration, gel filtration, SDS- 10 polyacrylamide gel electrophoresis, etc.; a method utilizing difference in electric charge such as ion exchange chromatography, etc.; a method utilizing difference in specific affinity such as affinity chromatography, etc.; a method utilizing difference in 15 hydrophobicity such as reverse phase high performance liquid chromatography, etc.; a method utilizing difference in isoelectric point such as isoelectrofocusing electrophoresis; and the like.

20 [0041]

When the receptor protein thus obtained is in a free form, it can be converted into the salt by publicly known methods or modifications thereof. On the other hand, when the receptor protein is obtained in 25 the form of a salt, it can be converted into the free form or in the form of a different salt by publicly known methods or modifications thereof.

The receptor protein produced by the recombinant can be treated, prior to or after the purification, 30 with an appropriate protein modifying enzyme so that the protein or partial peptide can be appropriately modified to partially remove a polypeptide. Examples of the protein-modifying enzyme include trypsin, chymotrypsin, arginyl endopeptidase, protein kinase, 35 glycosidase and the like.

The activity of the thus produced receptor protein of the present invention or salts thereof can be determined by a binding test to a labeled ligand and by an enzyme immunoassay using a specific antibody.

5

[0042]

Antibodies to the receptor protein, the partial peptide or salts according to the present invention may be any of polyclonal and monoclonal antibodies, so long 10 as they can recognize the receptor protein, the partial peptide or salts of the present invention.

The antibody to the receptor protein, the partial peptide or salts thereof (hereinafter refer to as receptor protein or the like of the present invention) 15 can be manufactured by publicly known methods for manufacturing antibodies or antisera, using as an antigen the receptor protein or the like of the present invention.

20

[0043]

[Preparation of monoclonal antibody]

(a) Preparation of monoclonal antibody-producing cells

The receptor protein or the like of the present invention is administered to a mammal, either solely or 25 together with carriers or diluents to the site that can produce the antibody by the administration. In order to potentiate the antibody productivity upon the administration, complete Freund's adjuvant or incomplete Freund's adjuvant may be administered. The 30 administration is effected usually once every 2 to 6 weeks and approximately 2 to 10 times in total. The mammals to be used include monkey, rabbit, dog, guinea pig, mouse, rat, sheep, goat and the like, with mouse and rat being preferred.

In the preparation of the monoclonal antibody-producing cells, an animal wherein the antibody titer is noted is selected from warm-blooded animals immunized with antigens, e.g., mice, then spleen or 5 lymph node is collected after two to five days from the final immunization and the antibody-producing cells contained therein are fused with myeloma cells to give monoclonal antibody-producing hybridomas. The antibody titer in antisera may be determined, for example, by 10 reacting the labeled receptor protein, which will be described later, with the antiserum followed by measuring the binding activity of the labeling agent bound to the antibody. The fusion may be carried out, for example, according to the method for Koehler and 15 Milstein (Nature, 256, 495, 1975). Examples of the fusion accelerator are polyethylene glycol (PEG), Sendai virus, etc. and PEG is preferably used.

Examples of myeloma cells include NS-1, P3U1 and SP2/0, with P3U1 being preferred. The ratio of the 20 number of the antibody-producing cells (spleen cells) to the number of myeloma cells to be used is preferably about 1:1 to about 20:1 and PEG (preferably PEG 1000 to PEG 6000) is added in a concentration of about 10% to about 80%. The cell fusion can be efficiently carried 25 out by incubating both cells at about 20°C to about 40°C, preferably about 30°C to about 37°C for about 1 minute to about 10 minutes.

[0044]

30 Various methods can be used for screening of a monoclonal antibody-producing hybridoma. Examples of such methods include a method which comprises adding the supernatant of hybridoma to a solid phase (e.g., microplate) adsorbed with the receptor protein or the 35 like as antigen directly or together with a carrier,

adding an anti-immunoglobulin antibody (where mouse cells are used for the cell fusion, anti-mouse immunoglobulin antibody is used) labeled with a radioactive substance or an enzyme or Protein A and
5 detecting the monoclonal antibody bound to the solid phase, and a method which comprises adding the supernatant of hybridoma to a solid phase adsorbed with an anti-immunoglobulin antibody or Protein A, adding the receptor protein or the like labeled with a
10 radioactive substance or an enzyme and detecting the monoclonal antibody bound to the solid phase.

The monoclonal antibody can be selected according to publicly known methods or their modifications. In general, the selection can be effected in a medium for
15 animal cells supplemented with HAT (hypoxanthine, aminopterin and thymidine). Any selection and growth medium can be employed as far as the hybridoma can grow therein. For example, RPMI 1640 medium containing 1% to 20%, preferably 10% to 20% fetal bovine serum, GIT
20 medium (Wako Pure Chemical Industries, Ltd.) containing 1% to 10% fetal bovine serum, a serum free medium for cultivation of a hybridoma (SFM-101, Nissui Seiyaku Co., Ltd.) and the like can be used for the selection and growth medium. The cultivation is carried out generally
25 at 20°C to 40°C, preferably 37°C, for about 5 days to about 3 weeks, preferably 1 to 2 weeks, normally in 5% CO₂. The antibody titer of the culture supernatant of a hybridoma can be determined as in the determination of antibody titer in antisera described above.

30

[0045]

(b) Purification of monoclonal antibody

Separation and purification of a monoclonal antibody can be carried out according the same manner
35 as applied to conventional separation and purification

for polyclonal antibodies, such as separation and purification of immunoglobulins (for example, salting-out, alcohol precipitation, isoelectric point precipitation, electrophoresis, adsorption and desorption with ion exchangers (e.g., DEAE), ultracentrifugation, gel filtration, or a specific purification method which comprises collecting only an antibody with an activated adsorbent such as an antigen-binding solid phase, Protein A or Protein G and dissociating the binding to obtain the antibody.

[0046]

[Preparation of polyclonal antibody]

The polyclonal antibody of the present invention can be manufactured by publicly known methods or modifications thereof. For example, a complex of immunogen (antigen of the receptor protein or the like) and a carrier protein is formed and a mammal is immunized with the complex in a manner similar to the method described above for the manufacture of monoclonal antibody. The product containing the antibody to the receptor protein or the like of the present invention is collected from the immunized animal followed by separation and purification of the antibody.

In the complex of immunogen and carrier protein for immunizing mammals, the type of carrier protein and the mixing ratio of carrier to hapten may be any type and in any ratio, as long as the antibody is efficiently produced to the hapten immunized by crosslinking to the carrier. For example, bovine serum albumin, bovine thyroglobulin or keyhole limpet hemocyanin is coupled to hapten in a carrier-to-hapten weight ratio of approximately 0.1 to 20, preferably 1 to 5.

A variety of condensation agents can be used for the coupling of carrier to hapten. Glutaraldehyde, carbodiimide, maleimide activated ester and activated ester reagents containing thiol group or dithiopyridyl 5 group are used for the coupling.

The condensation product is administered to warm-blooded animals either solely or together with carriers or diluents to the site that can produce the antibody by the administration. In order to potentiate the 10 antibody productivity upon the administration, complete Freund's adjuvant or incomplete Freund's adjuvant may be administered. The administration is usually carried out once every 2 to 6 weeks and 3 to 10 times in total.

The polyclonal antibody can be collected from the 15 blood, ascites, etc., preferably from the blood of mammals immunized by the method described above.

The polyclonal antibody titer in antiserum can be determined by the same procedure as in the serum antibody titer described above. The polyclonal 20 antibody can be separated and purified according to the same method for separation and purification of immunoglobulin as used for the monoclonal antibody described above.

25 [0047]

The receptor protein of the present invention, the partial peptide, or salts thereof and the DNA coding the same can be used: (1) for determination of ligand (agonist) to the receptor protein of the present 30 invention, (2) as an agent for the prevention and/or treatment of disease associated with dysfunction of the G protein coupled receptor protein of the present invention, (3) as a genetic diagnostic agent, (4) for quantification of ligand to the G protein coupled 35 receptor protein of the present invention, (5) for

screening of a compound (agonist, antagonist, etc.) that alters the binding property between the G protein coupled receptor protein of the present invention and a ligand, (6) as the agent for the prevention and/or 5 treatment of various diseases, comprising a compound (agonist, antagonist, etc.) that alters the binding property between the G protein coupled receptor protein of the present invention and a ligand, (7) for the quantification of the receptor protein, the partial 10 peptide or salts of the present invention, (8) for the neutralization by antibodies to the receptor protein, the partial peptide or salts of the present invention and (9) for preparation of non-human animal bearing the DNA encoding the G protein coupled receptor protein of 15 the present invention.

In particular, a compound (e.g., agonist, antagonist, etc.) that alters the binding property between a ligand and the G protein coupled receptor protein specific to human or other mammals can be 20 screened, using the receptor binding assay system by applying thereto the expression system of the recombinant G protein coupled receptor protein of the present invention. The agonist or antagonist can be used as a prophylactic/therapeutic agent for various 25 diseases.

The receptor protein of the present invention, the partial peptide or salts thereof (hereinafter, the receptor protein or the like of the present invention), DNA encoding the receptor protein or the partial 30 peptide of the present invention (hereinafter sometimes collectively referred to as the DNA of the present invention) and antibodies to the receptor protein or the like of the present invention (hereinafter sometimes referred to as the antibody of the present 35 invention) are specifically described with their use.

[0048]

(1) Determination of a ligand (agonist) to the G protein coupled receptor protein of the present
5 invention

The receptor protein or salt of the present invention or the partial peptide or salt of the present invention is useful as a reagent for searching and determining a ligand (agonist) to the receptor protein
10 of the present invention and salts thereof.

That is, the present invention provides a method for determining a ligand to the receptor protein or salt of the present invention or the partial peptide or salts thereof, which comprises bringing the receptor
15 protein of the present invention in contact with a test compound.

Examples of compounds to be tested include publicly known ligands (e.g., angiotensin, bombesin, canavinoïd, cholecystokinin, glutamine, serotonin,
20 melatonin, neuropeptide Y, opioid, purines, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedulin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, vasoactive intestinal and related polypeptide (VIP), somatostatin, dopamine, motilin, amylin, bradykinin,
25 calcitonin gene-related peptide (CGRP), leukotrienes, pancreastatin, prostaglandins, thromboxane, adenosine, adrenaline, α and β -chemokines (e.g., IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP-1 α , MIP-1 β , RANTES, etc.), endothelin,
30 enterogastrin, histamin, neurotensin, TRH, pancreatic polypeptide or galanin, etc.) as well as other substances, for example, tissue extracts and cell culture supernatants from humans and mammals (e.g., mice, rats, swine, bovine, sheep, monkeys). For
35 example, the tissue extract or cell culture supernatant

is added to the receptor protein of the present invention and fractionated while assaying the cell-stimulating activities to finally give a single ligand.

5 [0049]

In more detail, the method for determining a ligand of the present invention comprises determining compounds (e.g., peptides, proteins, non-peptide compounds, synthetic compounds, fermentation products) or salts thereof that bind to the receptor protein of the present invention to provide cell stimulating activities (e.g., the activities that promote or suppress arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP production, intracellular cGMP production, inositol phosphate production, change in cell membrane potential, phosphorylation of intracellular proteins, activation of c-fos and pH reduction), using the receptor protein, the partial peptide or salts of the present invention, or using the constructed recombinant receptor protein expression system in the receptor binding assay.

The method for determining a ligand of the present invention is characterized, for example, by measurement of the amount of the test compound bound to the receptor protein or the partial peptide of the present invention or the cell-stimulating activity when the test compound is brought in contact with the receptor protein or the partial peptide of the present invention.

30 [0050]

More specifically, the present invention provides the following:

(1) A method for determining a ligand to the receptor protein of the present invention or salts thereof which comprises bringing a labeled test

compound in contact with the receptor protein or salt thereof, or the partial peptide or salt thereof of the present invention and measuring the amount of the labeled test compound bound to the receptor protein or 5 salt thereof, or the partial peptide or salt thereof;

(2) A method for determining a ligand to the receptor protein of the present invention or salts thereof which comprises bringing a labeled test compound in contact with cells containing the receptor 10 protein of the present invention or with a membrane fraction of the cells and measuring the amount of the labeled test compound bound to the cells or the membrane fraction;

(3) A method for determining a ligand to the receptor protein of the present invention which comprises culturing a transformant containing DNA encoding the receptor protein of the present invention, bringing a labeled test compound in contact with the receptor protein expressed on the cell membrane by said 20 culturing, and measuring the amount of the labeled test compound bound to the expressed receptor protein;

[0051]

(4) A method for determining a ligand to the receptor protein of the present invention and salts thereof which comprises bringing a test compound in contact with cells containing the receptor protein of the present invention and measuring the receptor protein-mediated cell stimulating activities (e.g., the 30 activities that promote or suppress arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP production, intracellular cGMP production, inositol phosphate production, change in cell membrane potential, phosphorylation of

intracellular proteins, activation of c-fos and pH reduction); and,

(5) A method for determining a ligand to the receptor protein of the present invention and salts thereof which comprises culturing a transformant containing DNA encoding the receptor protein of the present invention, bringing a labeled test compound in contact with the receptor protein expressed on the cell membrane by said culturing, and measuring the receptor protein-mediated cell stimulating activities (e.g., the activities that promote or suppress arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP production, intracellular cGMP production, inositol phosphate production, change in cell membrane potential, phosphorylation of intracellular proteins, activation of c-fos and pH reduction).

In particular, it is preferred to perform the methods (1) to (3) thereby to confirm that a test compound can bind to the receptor protein of the present invention, followed by the methods (4) and (5).

[0052]

Any protein exemplified to be usable as the receptor protein can be used for the method for the present invention for determining ligands. However, the receptor protein that is abundantly expressed using animal cells is appropriate for the present invention.

The receptor protein of the present invention can be manufactured by the method for expression described above, preferably by expressing DNA encoding the receptor protein in mammalian or insect cells. DNA fragments encoding the desired portion of the protein include, but are not limited to, complementary DNA. For example, gene fragments or synthetic DNA may also be

used. For introducing a DNA fragment encoding the receptor protein or the like of the present invention into host animal cells and efficiently expressing the same, it is preferred to insert the DNA fragment downstream the polyhedrin promoter of nuclear polyhedrosis virus (NPV), which is a baculovirus having insect hosts, an SV40-derived promoter, a retrovirus promoter, a metallothionein promoter, a human heat shock promoter, a cytomegalovirus promoter, an SR α promoter or the like. The amount and quality of the receptor expressed can be determined by a publicly known method. For example, this determination can be made by the method described in the literature (Nambi, P. et al., J. Biol. Chem., Vol. 267, pp. 19555-19559 (1992)).

[0053]

Accordingly, the subject containing the receptor protein, the partial peptide or salts thereof in the method of the present invention for determining the ligand may be the receptor protein, the partial peptide or salts thereof purified by publicly known method, cells containing the receptor protein or membrane fraction of such cells.

Where cells containing the receptor protein of the present invention are used for the method for the present invention for determination of ligands, the cells may be fixed using glutaraldehyde, formalin etc. The fixation can be made by a publicly known method.

Cells containing the receptor protein of the present invention are host cells that have expressed the receptor protein of the present invention, which host cells include Escherichia coli, Bacillus subtilis, yeast, insect cells and animal cells.

The cell membrane fraction is a fraction abundant in cell membrane obtained by cell disruption and subsequent fractionation by a publicly known method. Useful cell disruption methods include cell squashing 5 using a Potter-Elvehjem homogenizer, disruption using a Waring blender or Polytron (manufactured by Kinematica Inc.), disruption by ultrasonication, and disruption by cell spraying via a thin nozzle under increased pressure using a French press or the like. Cell 10 membrane fractionation is effected mainly by fractionation using a centrifugal force, such as centrifugation for fractionation and density gradient centrifugation. For example, cell disruption fluid is centrifuged at a low rate (500 to 3,000 rpm) for a 15 short period of time (normally about 1 to 10 minutes), the resulting supernatant is then centrifuged at a higher rate (15,000 to 30,000 rpm) normally for 30 minutes to 2 hours. The precipitate thus obtained is used as the membrane fraction. The membrane fraction is 20 rich in the receptor protein expressed and membrane components such as cell-derived phospholipids and membrane proteins.

[0054]

25 The amount of the receptor protein in the cells containing the receptor protein and in the membrane fraction is preferably 10^3 to 10^8 molecules per cell, more preferably 10^5 to 10^7 molecules per cell. As the amount of expression increases, the ligand binding 30 activity per unit of membrane fraction (specific activity) increases so that not only the highly sensitive screening system can be constructed but also large quantities of samples can be assayed with the same lot.

To perform the methods (1) through (3) for determination of a ligand to the receptor protein of the present invention or salts thereof, an appropriate receptor fraction and a labeled test compound are
5 required.

The receptor protein fraction is preferably a fraction of naturally occurring receptor protein or a recombinant receptor fraction having an activity equivalent to that of the natural protein. Herein, the
10 term "equivalent activity" is intended to mean a ligand binding activity or a signal transduction activity that is equivalent to that possessed by naturally occurring receptor proteins.

Preferred examples of labeled test compounds
15 include angiotensin, bombesin, canavinoind, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purines, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedulin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, vasoactive
20 intestinal polypeptide (VIP), somatostatin, dopamine, motilin, amylin, bradykinin, calcitonin gene-related peptide (CGRP), leukotrienes, pancreastatin, prostaglandins, thromboxane, adenosine, adrenaline, α and β -chemokines (e.g., IL-8, GRO α , GRO β , GRO γ , NAP-2,
25 ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP-1 α , MIP-1 β , RANTES, etc.), endothelin, enterogastrin, histamin, neuropeptid Y, TRH, pancreatic polypeptide or galanin, etc.), which are labeled with [3 H], [125 I], [14 C], [35 S], etc.

30

[0055]

More specifically, the ligand to the receptor protein or salt of the present invention is determined by the following procedures. First, a standard receptor
35 preparation is prepared by suspending cells containing

the receptor protein of the present invention or the membrane fraction thereof in a buffer appropriate for use in the determination method. Any buffer can be used so long as it does not interfere with ligand-receptor binding, such buffers including a phosphate buffer or a Tris-HCl buffer having pH of about 4 to 10 (preferably pH of about 6 to 8). For the purpose of minimizing non-specific binding, a surfactant such as CHAPS, Tween-⁸⁰™ (manufactured by Kao-Atlas Inc.), digitonin or deoxycholate, and various proteins such as bovine serum albumin or gelatin, may optionally be added to the buffer. Further for the purpose of suppressing the degradation of the receptor or ligand by protease, a protease inhibitor such as PMSF, leupeptin, E-64 (manufactured by Peptide Institute, Inc.) and pepstatin may also be added. A given amount (5,000 to 500,000 cpm) of the test compound labeled with [³H], [¹²⁵I], [¹⁴C], [³⁵S] or the like is added to 0.01-10 ml of the receptor solution. To determine the amount of non-specific binding (NSB), a reaction tube containing an unlabeled test compound in a large excess is also provided. The reaction is carried out at approximately 0 to 50°C, preferably about 4 to 37°C for about 20 minutes to about 24 hours, preferably about 30 minutes to 3 hours. After completion of the reaction, the reaction mixture is filtrated through glass fiber filter paper etc. and washed with an appropriate amount of the same buffer. The residual radioactivity in the glass fiber filter paper is then measured by means of a liquid scintillation counter or γ -counter. A test compound exceeding 0 cpm in count obtained by subtracting nonspecific binding (NSB) from the total binding (B) (B minus NSB) may be selected as a ligand (agonist) to the receptor protein of the present invention or salts thereof.

[0056]

The method (4) or (5) above for determination of a ligand to the receptor protein of the present invention or salts thereof can be performed as follows. The receptor protein-mediated cell-stimulating activities (e.g., the activities that promote or suppress arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP production, intracellular cGMP production, inositol phosphate production, change in cell membrane potential, phosphorylation of intracellular proteins, activation of c-fos and pH reduction) may be determined by a publicly known method, or using an assay kit commercially available. Specifically, cells containing the receptor protein are first cultured on a multiwell plate, etc. Prior to the ligand determination, the medium is replaced with fresh medium or with an appropriate non-cytotoxic buffer, followed by incubation for a given period of time in the presence of a test compound, etc. Subsequently, the cells are extracted or the supernatant is recovered and the resulting product is quantified by appropriate procedures. Where it is difficult to detect the production of the index substance for the cell-stimulating activity (e.g., arachidonic acid) due to a degrading enzyme contained in the cells, an inhibitor against such a degrading enzyme may be added prior to the assay. For detecting activities such as the cAMP production suppression activity, the baseline production in the cells is increased by forskolin or the like and the suppressing effect on the increased baseline production can then be detected.

The kit for determination of a ligand that binds to the receptor protein or salt of the present invention comprises the receptor protein or salt of the present invention, the partial peptides or salt of the 5 present invention, cells containing the receptor protein of the present invention, or the membrane fraction of the cells containing the receptor protein of the present invention.

Examples of the ligand determination kit of the 10 present invention are given below.

1. Reagents for determining ligands

(1) Buffers for assay and washing

Hanks' Balanced Salt Solution (manufactured by 15 Gibco Co.) supplemented with 0.05% bovine serum albumin (Sigma Co.).

The solution is sterilized by filtration through a 0.45 μm filter and stored at 4°C. Alternatively, the solution may be prepared at use.

20 (2) Standard G protein coupled receptor protein

CHO cells on which the receptor protein of the present invention has been expressed are subjected to passage culture in a 12-well plate in a density of 5×10^5 cells/well followed by culturing at 37°C under 5% CO₂ and 95% air for two days.

25 (3) Labeled test compounds

Compounds labeled with commercially available [³H], [¹²⁵I], [¹⁴C] or [³⁵S] or compounds labeled by appropriate methods.

30 An aqueous solution of the compound is stored at 4°C or -20°C. The solution is diluted to 1 μM with an assay buffer at use. A sparingly water-soluble test compound is dissolved in dimethylformamide, DMSO or methanol.

35 (4) Non-labeled compounds

A non-labeled form of the same compound as the labeled compound is prepared in a concentration 100 to 1,000-fold higher than that of the labeled compound.

5 [0058]

2. Method for assay

(1) CHO cells expressing the receptor protein of the present invention are cultured in a 12-well culture plate. After washing twice with 1 ml of an assay buffer, 10 490 µl of the assay buffer is added to each well.

(2) After 5e µl of the labeled test compound is added, the resulting mixture is incubated at room temperature for an hour. To determine the non-specific binding, 5 µl of the non-labeled compound is added to 15 the system.

(3) The reaction mixture is removed and the wells are washed 3 times with 1 ml of washing buffer. The labeled test compound bound to the cells is dissolved in 0.2N NaOH-1% SDS and then mixed with 4 ml of liquid 20 scintillator A (manufactured by Wako Pure Chemical Industries, Ltd.).

(4) The radioactivity is measured using a liquid scintillation counter (manufactured by Beckman Co.).

25 [0059]

The ligands that bind to the receptor protein or salt of the present invention include substances specifically present in the brain, pituitary gland and pancreas. Examples of such ligands are angiotensin, 30 bombesin, canavinoïd, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioids, purines, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedulin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, vasoactive intestinal peptide (VIP), 35 somatostatin, dopamine, motilin, amylin, bradykinin,

calcitonin gene-related peptide (CGRP), leukotriens, pancreastatin, prostaglandins, thromboxane, adenosine, adrenaline, α and β -chemokines (e.g. IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, 5 MCP-3, I-309, MIP1 α , MIP-1 β , and RANTES), endothelin, enterogastrin, histamine, neuropeptides, TRH, pancreatic polypeptide and galanin.

[0060]

10 (2) Prophylactic and/or therapeutic agents for diseases associated with the dysfunction of the G protein coupled receptor protein of the present invention
In the method according to (1), if the ligand to the receptor protein of the present invention is identified, (i) the receptor protein of the present invention or (ii) the DNA encoding the receptor protein can be used as a prophylactic and/or therapeutic agents for diseases associated with the dysfunction of the ligand peptide of the present invention depending on 15 the functions that the ligand has.
20

For example, when any physiological activity of the receptor protein of the present invention cannot be expected due to a reduced level of the receptor protein in a patient (deficiency of the receptor protein), the 25 ligand activity can be exhibited by the following methods: (1) the receptor protein of the present invention is administered to the patient to supplement the amount of the receptor protein; or (2) the amount of the receptor protein of the present invention is increased in the patient by: a) administration of the 30 DNA encoding the receptor protein of the present invention to the patient for expression, or by b) insertion of the DNA encoding the receptor protein of the present invention in the target cells for expression, and the cells thus expressed are then 35

transplanted into the patient. Thus, the amount of the receptor protein of the present invention can be increased in the patient, whereby the ligand activity can be exhibited sufficiently. Therefore, the DNA 5 encoding the receptor protein of the present invention is useful as a safe and low toxic prophylactic and/or therapeutic drug for diseases associated with the dysfunction of the receptor protein of the present invention.

10 The receptor protein of the present invention has about 35% of homology to the galanin receptor, which is one of the G protein coupled receptor proteins, with regard to the amino acid sequence. The galanin receptor is expressed in central nervous system, pituitary gland, peripheral nervous system, digestive tract, urogenital system, suprarenalis, respiratory apparatus and pancreas [*Gendai no sinkei-naibunpitsugaku* (Modern neuroendocrinology) supervised by N. Yoshida, pp.111 - 116 (1996)]. With regard to 15 the disorders, there are athrocytosis, dysmnesia or prolactin hyper- or hyposecretion in central nervous system. Further, there are suppression of insulin secretion, sthenia of growth hormone secretion, analgetic function, suppression of gastlin secretion, 20 suppression of enterokinesis in peripheral system [Critical Reviews in Neurobiology, 7(3/4): 229 - 274(1993)]. Therefore, the receptor of the present invention homologous to the galanin receptor is useful for Prophylaxis and/or therapy for central disorders 25 such as Alzheimer's disease, dementia or feeding disorder, secretion disorders in lever, cholecyst, pancreas or internal secretion such as diabetes or feeding disorder, or disease of cardiovascular system such as hypertension, hypercardia, stenocardia or 30 arteriosclerosis.

Where the receptor protein or the like of the present invention is used as a prophylactic/therapeutic agent as described above, the receptor protein or the like can be prepared into a pharmaceutical preparation 5 by a publicly known method.

When the DNA encoding the receptor protein of the present invention (hereinafter sometimes referred to as the DNA of the present invention) is used as a prophylactic/therapeutic agent as described above, the 10 DNA of the present invention may be used alone or after inserting it into an appropriate vector such as retrovirus vector, adenovirus vector or adenovirus-associated virus vector followed by a conventional means for drug administration. The DNA of the present 15 invention can also be administered as naked DNA, or with adjuvants to assist its uptake by gene gun or through a catheter such as a catheter with a hydrogel.

For example, (1) the receptor protein of the present invention or (2) the DNA encoding the receptor 20 protein can be used orally in the form of tablets which may be sugar coated if necessary and desired, capsules, elixirs, microcapsules etc., or parenterally in the form of injectable preparations such as a sterile solution and a suspension in water or with other 25 pharmaceutically acceptable liquid. These preparations can be manufactured by mixing (1) the receptor protein of the present invention or (2) the DNA encoding the receptor protein with a physiologically acceptable known carrier, flavoring agent, excipient, vehicle, 30 antiseptic, stabilizer, binder, etc. in a unit dosage form required in a generally accepted manner applied to making pharmaceutical preparations. The active ingredient in the preparation is controlled in such a dose that an appropriate dose is obtained within the 35 specified range given.

[0061]

Additives miscible with tablets, capsules etc. include a binder such as gelatin, corn starch, 5 tragacanth and gum arabic, an excipient such as crystalline cellulose, a swelling agent such as corn starch, gelatin and alginic acid, a lubricant such as magnesium stearate, a sweetening agent such as sucrose, lactose and saccharin, and a flavoring agent such as peppermint, akamono oil and cherry. When the unit 10 dosage is in the form of capsules, liquid carriers such as oils and fats may further be used together with the additives described above. A sterile composition for injection may be formulated according to a conventional manner used to make pharmaceutical compositions, e.g., 15 by dissolving or suspending the active ingredients in a vehicle such as water for injection with a naturally occurring vegetable oil such as sesame oil and coconut oil, etc. to prepare the pharmaceutical composition.

20 Examples of an aqueous medium for injection include physiological saline and an isotonic solution containing glucose and other auxiliary agents (e.g., D-sorbitol, D-mannitol and sodium chloride) and may be used in combination with an appropriate dissolution aid 25 such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol and polyethylene glycol), a nonionic surfactant (e.g., polysorbate 80TM and HCO-50), etc. As an oily medium, for example, sesame oil and soybean oil may be used, which can be used in combination with a 30 dissolution aid such as benzyl benzoate and benzyl alcohol.

[0062]

Furthermore the prophylactic/therapeutic agent 35 described above may also be formulated with a buffer

(e.g., phosphate buffer and sodium acetate buffer) a soothing agent (e.g., benzalkonium chloride, procaine hydrochloride), a stabilizer (e.g., human serum albumin, polyethylene glycol), a preservative (e.g., benzyl alcohol, phenol), an antioxidant, etc. The thus-prepared liquid for injection is normally filled in an appropriate ampoule.

Since the thus obtained pharmaceutical preparation is safe and low toxic, the preparation can be administered to human or mammals (e.g., rat, mouse, rabbit, sheep, swine, bovine, cat, dog, monkey, etc.).

The dose of the receptor protein of the present invention varies depending on subject to be administered, target organ, symptom, route for administration, etc.; in oral administration, the dose is normally about 0.1 to about 100 mg, preferably about 1.0 to about 50 mg, and more preferably about 1.0 to about 20 mg per day for a patient with Alzheimer's disease (weighing 60 kg). In parenteral administration, the single dose varies depending on subject to be administered, target organ, symptom, route for administration, etc. but it is advantageous to administer the active ingredient intravenously at a daily dose of about 0.01 to about 30 mg, preferably about 0.1 to about 20 mg, and more preferably about 0.1 to about 10 mg for a patient with Alzheimer's disease (weighing 60 kg). For other animal species, the corresponding dose as converted per 60 kg weight can be administered.

The dose of the DNA of the present invention varies depending on subject to be administered, target organ, symptom, route for administration, etc.; in oral administration, the dose is normally about 0.1 to about 100 mg, preferably about 1.0 to about 50 mg, and more preferably about 1.0 to about 20 mg per day for a

patient with Alzheimer's disease (weighing 60 kg). In parenteral administration, the single dose varies depending on subject to be administered, target organ, symptom, route for administration, etc. but it is
5 advantageous to administer the active ingredient intravenously at a daily dose of about 0.01 to about 30 mg, preferably about 0.1 to about 20 mg, and more preferably about 0.1 to about 10 mg for a patient with Alzheimer's disease (weighing 60 kg). For other animal
10 species, the corresponding dose as converted per 60 kg weight can be administered.

[0063]

(3) Gene diagnostic agent

15 Using as a probe, an abnormality (gene abnormality) of the DNA encoding the receptor protein or the partial peptide of the present invention, or mRNA in human or mammal (e.g., rat, mouse, rabbit, sheep, swine, bovine, cat, dog, monkey, etc.) can be
20 detected. Therefore, the DNA of the present invention is useful as a gene diagnostic agent for the damage to the DNA or mRNA, mutation thereof, or decreased expression thereof, or increased expression or overexpression of the DNA or mRNA.

25 The gene diagnosis described above using the DNA of the present invention can be performed by, for example, publicly known Northern hybridization assay or PCR-SSCP assay (Genomics, 5, 874-879 (1989); Proceedings of the National Academy of Sciences of the
30 United States of America, 86, 2766-2770 (1989)).

[0064]

(4) Quantification of ligand to the G protein coupld receptor protein of the present invention

Since the receptor protein or the like of the present invention has a binding property to ligand, the ligand activity can be quantified in vivo with high sensitivity.

5 The method for quantification of the present invention may be effected, for example, in combination with a competitive method. Thus, a sample to be determined is brought into contact with the receptor protein of the present invention, whereby the ligand concentration in the sample can be determined.
10 Specifically, the quantification can be performed by the following method (1) or (2) below or its modification:

15 (1) Hiroshi Irie (ed.): "Radioimmunoassay" (1974, published by Kodansha, Japan); and

 (2) Hiroshi Irie (ed.): "Radioimmunoassay, Second Series" (1979, published by Kodansha, Japan.).

[0065]

20 (5) A method for screening of the compound (agonist, antagonist, etc.) that alters the binding property between the G protein coupled receptor protein and ligand

25 By using the receptor protein or the like of the present invention, or by constructing the expression system of recombinant receptor protein or the like and using the receptor-binding assay system via the expression system, screening can be made efficiently on the compound (e.g., peptide, protein, a non-peptide
30 compound, a synthetic compound, fermentation product, etc.) or salts thereof that alter the binding property between the ligand and the receptor protein or the like of the present invention.

 Examples of the above compounds include (a) a
35 compound exhibiting cell stimulating activities

mediated by the G protein coupled receptor (e.g., the activities that promote or suppress arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP production, intracellular 5 cGMP production, inositol phosphate production, change in cell membrane potential, phosphorylation of intracellular proteins, activation of c-fos and pH reduction) (so-called agonists to the receptor protein of the present invention), (b) a compound free of such 10 a cell stimulating activity (so-called antagonists to the receptor protein of the present invention); (c) a compound that increases the binding property between ligand and the G protein coupled receptor protein of the present invention or (c) a compound that decreases 15 the binding property between ligand and the G protein coupled receptor protein of the present invention (the compound (a) is screened preferably by the method for determination of ligand described above).

Thus, the present invention also provides a method 20 for screening a compound or a salt thereof that alters the binding property between the receptor protein, the partial peptide or the salts of the present invention and the ligand, which comprises comparing the following two cases: (i) the case wherein the receptor protein, 25 the partial peptides or the salts of the present invention is brought in contact with the ligand; and (ii) the case wherein the receptor protein, the partial peptides or the salts of the present invention is brought in contact with the ligand and a test compound.

In the screening method for the present invention, comparison is made between the cases (i) and (ii) in terms of, e.g., the amount of the ligand that binds to the receptor protein or the like, or the cell-stimulating activities.

[0066]

More specifically, the present invention provides the following methods.

(1) A method for screening a compound or a salt thereof that alters the binding property between a ligand and the receptor protein or the like of the present invention, which comprises measuring the amount of a labeled ligand bound to the receptor protein or the like of the present invention in the case wherein the labeled ligand is brought in contact with the receptor protein or the like of the present invention and in the case wherein the labeled ligand and a test compound are brought in contact with the receptor protein or the like, and comparing the binding amount of the labeled ligand between the two cases.

(2) A method for screening a compound or a salt thereof that alters the binding property between a ligand and the receptor protein or the like of the present invention, which comprises measuring the amount of a labeled ligand bound to cells containing the receptor protein or the like of the present invention or a cell fraction thereof, in the case wherein the labeled ligand is brought in contact with the cells containing the receptor protein or the like of the present invention or the cell membrane and in the case wherein the labeled ligand and a test compound are brought in contact with the cells containing the receptor protein or the like or the cell membrane, and comparing the binding amount of the labeled ligand between the two cases.

(3) A method for screening a compound or a salt thereof that alters the binding property between a ligand and the receptor protein or the like of the present invention, which comprises measuring the amount of a labeled ligand bound to the receptor protein or

the like of the present invention, in the case wherein
the labeled ligand is brought in contact with the
receptor protein or the like expressed in the cell
membrane by culturing a transformant containing the DNA
5 of the present invention and in the case wherein the
labeled ligand and a test compound are brought in
contact with the receptor protein or the like expressed
in the cell membrane by culturing a transformant
containing the DNA of the present invention, and
10 comparing the binding amount of the labeled ligand
between the two cases.

[0067]

(4) A method for screening a compound or a salt
15 thereof that alters the binding property between a
ligand and the receptor protein or the like of the
present invention, which comprises measuring receptor-
mediated cell stimulating activities (e.g., the
activities that promote or suppress arachidonic acid
20 release, acetylcholine release, intracellular Ca^{2+}
release, intracellular cAMP production, intracellular
cGMP production, inositol phosphate production, change
in cell membrane potential, phosphorylation of
intracellular proteins, activation of c-fos and pH
25 reduction) in the case wherein a compound (e.g., a
ligand to the receptor protein or the like of the
present invention) that activates the receptor protein
or the like of the present invention is brought in
contact with cells containing the receptor protein or
30 the like of the present invention and in the case
wherein said compound that activates the receptor
protein or the like of the present invention and a test
compound are brought in contact with the cells
containing the receptor protein or the like of the

present invention, and comparing the cell stimulating activities between the two cases.

(5) A method for screening a compound or a salt thereof that alters the binding property between a ligand and the receptor protein or the like of the present invention, which comprises measuring receptor-mediated cell stimulating activities (e.g., the activities that promote or suppress arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP production, intracellular cGMP production, inositol phosphate production, change in cell membrane potential, phosphorylation of intracellular proteins, activation of c-fos and pH reduction) in the case wherein a compound that activates the ligand peptide or the like of the present invention (e.g., a ligand to the receptor protein or the like of the present invention) is brought in contact with the receptor protein or the like of the present invention expressed in a cell membrane by culturing a transformant containing the DNA of the present invention and in the case wherein said compound that activates the receptor protein or the like of the present invention and a test compound are brought in contact with the receptor protein or the like of the present invention expressed in a cell membrane by culturing a transformant containing the DNA of the present invention, and comparing the cell stimulating activities between the two cases.

30 [0068]

Before the receptor protein or the like of the present invention was obtained, screening of the G protein coupled receptor agonist or antagonist should have been made in terms of whether or not a candidate compound would inhibit the binding between G protein

coupled receptor proteins and ligands, using cells, tissues or cell membrane fractions containing G protein coupled receptor proteins. When the cells, tissues or cell membrane fractions are used as they are, however, 5 other receptor proteins inevitably exist. It was thus difficult to screen agonists or antagonists to the desired receptor proteins.

However, the use of the receptor protein of the present invention enables to efficiently screen the 10 compound that inhibits the binding between a ligand and the G protein coupled receptor protein. Besides, it can be simply evaluated whether the compound screened is either an agonist or an antagonist.

Hereinafter the screening method for the present 15 invention will be described more specifically.

First, the receptor protein or the like of the present invention, which is used for the screening method for the present invention, may be any protein so long as it contains the receptor protein or the like of 20 the present invention described above, though a membrane fraction of mammalian organs is preferably employed. For screening that requires large quantities of the receptor protein, it is advantageous to use receptor proteins expressed abundantly by a recombinant.

25

[0069]

In the manufacture of the receptor protein or the like of the present invention, the methods described above can be used, though the DNA of the present 30 invention is preferably expressed in mammalian cells or insect cells. As the DNA fragment coding for the target protein region, complementary DNA may be used but is not limited thereto. For example, gene fragments or synthetic DNA may also be used as the DNA fragment. In 35 order to introduce the DNA fragment coding for the

receptor protein of the present invention into host animal cells and express it efficiently, the DNA fragment is preferably incorporated into a polyhedron promoter of nuclear polyhedrosis virus (NPV) belonging 5 to the baculovirus, a SV40-derived promoter, a promoter of retrovirus, a metallothionein promoter, a human heat shock promoter, a cytomegalovirus promoter, SR α promoter, etc. at the downstream thereof. The amount and quality of the thus expressed receptors can be 10 examined by a publicly known method, for example, by the method described in Nambi, P. et al., J. Biol. Chem., 267, 19555-19559 (1992).

Accordingly, in the screening method for the present invention, the substance containing the 15 receptor protein or the like of the present invention may be the receptor protein or the like that is purified by publicly known methods. Alternatively, cells containing the receptor protein or the like or a cell membrane fraction of the cells containing the 20 receptor protein or the like may be used as well.

[0070]

Where the cells containing the receptor protein or the like of the present invention are used in the 25 screening method for the present invention, these cells may be fixed with glutaraldehyde, formalin, etc. The fixation may be carried out by a publicly known method.

The cells containing the receptor protein or the like of the present invention refer to host cells expressing the receptor protein or the like. Examples 30 of such host cells include Escherichia coli, Bacillus subtilis, yeast, insect cells and animal cells.

The cell membrane fraction refers to a fraction that abundantly contains the cell membranes prepared by 35 a publicly known method after disrupting the cells.

Examples of cell disruption include cell squashing using a Potter-Elvehjem homogenizer, disruption using a Waring blender or Polytron (manufactured by Kinematica Inc.), disruption by ultrasonication, and disruption by cell spraying via a thin nozzle under increased pressure using a French press or the like. Cell membrane fractionation is effected mainly by fractionation using a centrifugal force, such as centrifugation for fractionation and density gradient centrifugation. For example, cell disruption fluid is centrifuged at a low rate (500 rpm to 3,000 rpm) for a short period of time (normally about 1 to 10 minutes), the resulting supernatant is then centrifuged at a higher rate (15,000 rpm to 30,000 rpm) normally for 30 minutes to 2 hours. The precipitate thus obtained is used as the membrane fraction. The membrane fraction is rich in the receptor protein or the like expressed and membrane components such as cell-derived phospholipids and membrane proteins.

The amount of the receptor protein contained in the cells containing the receptor protein or the like or in the membrane fraction is preferably 10^3 to 10^8 molecules per cell, more preferably 10^5 to 10^7 molecules per cell. As the amount of expression increases, the ligand binding activity per unit of membrane fraction (specific activity) increases so that not only the highly sensitive screening system can be constructed but also large quantities of samples can be assayed with the same lot.

30

[0071]

To perform the methods (1) through (3) for screening the compound that alters the binding property between the ligand and the receptor protein or the like

of the present invention, an appropriate receptor protein fraction and a labeled ligand are required.

The receptor protein fraction is preferably a fraction of naturally occurring receptor protein or a recombinant receptor protein fraction having an activity equivalent to that of the naturally occurring protein. Herein, the term "equivalent activity" is intended to mean a ligand binding activity or a signal transduction activity that is equivalent to that possessed by naturally occurring receptor proteins.

Examples of the labeled ligand include ligands that are labeled with [³H], [¹²⁵I], [¹⁴C] or [³⁵S].

More specifically, the compound that alters the binding property between the ligand and the receptor protein or the like of the present invention, is screened by the following procedures. First, a standard receptor preparation is prepared by suspending cells containing the receptor protein or the like of the present invention or the membrane fraction thereof in a buffer appropriate for use in the screening method. Any buffer can be used so long as it does not interfere the ligand-receptor binding. Examples of such buffers are a phosphate buffer or a Tris-HCl buffer, having pH of about 4 to 10 (preferably pH of about 6 to 8). For the purpose of minimizing non-specific binding, a surfactant such as CHAPS, Tween-80TM (Kao-Atlas Inc.), digitonin or deoxycholate may be added to buffers. Further for the purpose of suppressing the degradation of the receptor or ligand by protease, a protease inhibitor such as PMSF, leupeptin, E-64 (manufactured by Peptide Institute, Inc.) and pepstatin may also be added. A given amount (5,000 to 500,000 cpm) of the ligand labeled is added to 0.01 to 10 ml of the receptor solution, in which 10⁻⁴ M to 10⁻¹⁰ M of the test compound is co-present. To determine the amount of

non-specific binding (NSB), a reaction tube containing an unlabeled ligand in a large excess is also provided. The reaction is carried out at approximately 0 to 50°C, preferably about 4 to 37°C for about 20 minutes to 5 about 24 hours, preferably about 30 minutes to 3 hours. After completion of the reaction, the reaction mixture is filtrated through glass fiber filter paper, etc. and washed with an appropriate amount of the same buffer. The residual radioactivity in the glass fiber filter 10 paper is then measured by means of a liquid scintillation counter or γ -counter. When nonspecific binding (NSB) is subtracted from the count (B_0) where any antagonizing substance is absent and the resulting count (B_0 minus NSB) is made 100%, a test compound 15 showing the specific binding amount (B minus NSB) of, e.g., 50% or less may be selected as a candidate compound.

[0072]

20 The method (4) or (5) above for screening the compound that alters the binding property between the ligand and the receptor protein or the like of the present invention can be performed as follows. In one embodiment, the receptor protein-mediated cell 25 stimulating activities (e.g., the activities that promote or suppress arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP production, intracellular cGMP production, inositol phosphate production, change in 30 cell membrane potential, phosphorylation of intracellular proteins, activation of c-fos and pH reduction) may be determined by a publicly known method, or using an assay kit commercially available.

Specifically, the cells containing the receptor 35 protein or the like of the present invention are first

cultured on a multiwell plate, etc. Prior to screening, the medium is replaced with fresh medium or with an appropriate non-cytotoxic buffer, followed by incubation for a given period of time in the presence 5 of a test compound, etc. Subsequently, the cells are extracted or the supernatant is recovered and the resulting product is quantified by appropriate procedures. Where it is difficult to detect the production of the cell-stimulating activity index 10 substance (e.g., arachidonic acid) due to a degrading enzyme contained in the cells, an inhibitor against such a degrading enzyme may be added prior to the assay. For detecting activities such as the cAMP production suppression activity, the baseline production in the 15 cells is increased by forskolin or the like and the suppressing effect on the increased baseline production can then be detected.

For screening through the measurement of the cell stimulating activities, cells in which a appropriate 20 receptor protein is expressed are necessary. Preferred cells in which the receptor protein or the like of the present invention is expressed are a naturally occurring cell line containing the receptor protein or the like of the present invention and the aforesaid 25 cell line in which recombinant type receptor protein or the like is expressed.

Examples of the test compound include peptides, proteins, non-peptide compounds, synthetic compounds, fermentation products, cell extracts, plant extracts 30 and animal tissue extracts. These test compounds may be either novel or publicly known.

[0073]

A kit for screening the compound or a salt thereof 35 that alters the binding property between the ligand and

the receptor protein or the like of the present invention comprises the receptor protein or the like of the present invention, cells containing the receptor protein or the like of the present invention, or a 5 membrane fraction of the cells containing the receptor protein or the like of the present invention.

Examples of the screening kit include as follows:

1. Reagent for screening

(1) Buffers for assay and washing

10 Hanks' Balanced Salt Solution (manufactured by Gibco) supplemented with 0.05% of bovine serum albumin (manufactured by Sigma).

15 The buffers may be sterilized by filtration through a membrane filter with a 0.45 μm pore size and stored at 4 °C, or may be prepared at use.

(2) G protein coupled receptor protein preparation

20 CHO cells in which the receptor protein of the present invention is expressed are subcultured at 5 x 10⁵ cells/well on a 12-well plate followed by culturing at 37 °C under a 5% CO₂ and 95% air for 2 days.

(3) Labeled ligand

The ligand which is labeled with commercially available [³H], [¹²⁵I], [¹⁴C], [³⁵S], etc.

25 The product in the form of an aqueous solution is stored at 4 °C or at -20 °C and diluted to 1 μM with a buffer for the assay at use.

(4) Standard ligand solution

30 Ligand is dissolved in PBS containing 0.1% of bovine serum albumin (manufactured by Sigma) to make 1 mM and stored at -20 °C.

[0074]

2. Method for assay

35 (1) CHO cells are cultured in a 12-well tissue culture plate to express the receptor protein of the

present invention. After washing the receptor protein-expressing CHO cells twice with 1 ml of buffer for the assay, 490 μ l of the buffer for assay is added to each well.

5 (2) After 5 μ l of a test compound solution of 10^{-3} to 10^{-10} M is added, 5 μ l of a labeled ligand is added to the system followed by incubating at room temperature for an hour. To determine the amount of the non-specific binding, 5 μ l of the ligand of 10^{-3} M is
10 added to the system, instead of the test compound.

15 (3) The reaction mixture is removed from the well, which is washed three times with 1 ml each of the buffer for assay. The labeled ligand bound to the cells is dissolved in 0.2N NaOH-1% SDS and mixed with 4 ml of a liquid scintillator A (manufactured by Wako Pure
Chemical, Japan).

20 (4) Radioactivity is measured using a liquid scintillation counter (manufactured by Beckmann) and PMB (percent of the maximum binding) is calculated in accordance with the following equation:

[0075]

$$\text{PMB} = [(B - \text{NSB}) / (B_0 - \text{NSB})] \times 100$$

25 wherein:

PMB: percent of the maximum binding

B: value when a sample is added

NSB: non-specific binding

B_0 : maximum binding

30

[0076]

The compound or a salt thereof obtainable by the screening method or by the screening kit of the present invention is a compound that functions to alter the
35 binding property between the ligand and the receptor

protein or the like of the present invention. More specifically, the compound includes (a) a compound exhibiting cell stimulating activities mediated by the G protein coupled receptor (e.g., the activities that 5 promote or suppress arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP production, intracellular cGMP production, inositol phosphate production, change in cell membrane potential, phosphorylation of 10 intracellular proteins, activation of c-fos and pH reduction) (so-called agonists to the receptor protein of the present invention), (b) a compound free of such a cell stimulating activity (so-called antagonists to the receptor protein of the present invention); (c) a 15 compound that increases the binding property between the ligand and the G protein coupled receptor protein of the present invention and (d) a compound that decreases the binding property between the ligand and the G protein coupled receptor protein of the present 20 invention.

Examples of such compounds include peptides, proteins, non-peptide compounds, synthetic compounds and fermentation products. These compounds may be either novel or publicly known.

25 The agonist to the receptor protein or the like of the present invention has the same physiological activity as that of the ligand to the receptor protein or the like of the present invention. Therefore, the agonist is useful as a safe and low toxic 30 pharmaceutical, depending upon the ligand activity.

The agonist to receptor protein or the like of the present invention also possesses a placenta function regulating activity and are thus useful for prophylactic or therapeutic drug of cilia cancer, 35 hydatid mole, invasive mole, miscarriage, fetal

hypoplasia, sugar dysbolism, lipid dysbolism or labor induction.

The antagonist to the receptor protein or the like of the present invention can suppress the physiological activity that the ligand to the receptor protein or the like of the present invention has. Therefore, the antagonist is useful as a safe and low toxic pharmaceutical for suppressing the ligand activity.

The compound that increases the binding between the ligand and the receptor protein of the present invention is useful as a safe and low toxic pharmaceutical for decreasing the physiological activity that the ligand to the receptor protein or the like of the present invention has.

The compound that decreases the binding between the ligand and the receptor protein of the present invention is useful as a safe and low toxic pharmaceutical for decreasing the physiological activity that the ligand to the receptor protein or the like of the present invention has.

[0077]

When the compound or a salt thereof obtainable by the screening method or by the screening kit of the present invention is used as the pharmaceutical composition described above, a conventional means may be applied to making the composition. For example, the compound or a salt thereof may be prepared into tablets, capsules, elixirs, microcapsules, sterile solutions, suspensions, etc.

Since the thus obtained preparation is safe and low toxic, it can be administered to human or mammals (e.g., rat, mouse, rabbit, sheep, swine, bovine, cat, dog, monkey, etc.).

The dose of the compound or a salt thereof varies depending on subject to be administered, target organ, symptom, method for administration, etc.; in oral administration, the dose is normally about 0.1 to about 5 100 mg, preferably about 1.0 to about 50 mg, more preferably about 1.0 to about 20 mg per day for a patient with Alzheimer's disease (weighing 60 kg). In parenteral administration, the single dose varies depending on subject to be administered, target organ, 10 symptom, method for administration, etc. but it is advantageous to administer the active ingredient intravenously at a daily dose of about 0.01 to about 30 mg, preferably about 0.1 to about 20 mg, more preferably about 0.1 to about 10 mg for a patient with 15 Alzheimer's disease (weighing 60 kg). For other animal species, the corresponding dose as converted per 60 kg weight can be administered.

[0078]

20 (6) A composition for the prophylactic and/or therapy for various diseases comprising the compound (agonist, antagonist) that alters the binding property between the G protein coupled receptor protein or the like of the present invention and 25 the ligand

As stated hereinabove, there is a possibility that the receptor protein or the like of the present invention plays some important role in vivo, such as a central function. Therefore, the compound (agonist, 30 antagonist) that alter the binding property between the receptor protein of the present invention and the ligand can be used for the prophylactic and/or therapeutic agent of diseases associated with dysfunction of the receptor protein of the present 35 invention.

When the compound above is used as the pharmaceutical composition for the prevention and/or treatment of diseases associated with dysfunction of the receptor protein of the present invention, a 5 conventional means may be applied to making the composition.

For example, the compound may be prepared into a sugar coated tablet, a capsule, an elixir or a microcapsule for oral administration and for parenteral 10 administration in the form of injectable preparations such as a sterile solution and a suspension in water or with other pharmaceutically acceptable liquid. These preparations can be manufactured by mixing the compound with a physiologically acceptable known carrier, 15 flavoring agent, excipient, vehicle, antiseptic, stabilizer, binder, etc. in a unit dosage form required in a generally accepted manner for making pharmaceutical preparations. The active ingredient in the preparation is controlled in such a dose that an 20 appropriate dose is obtained within the specified range given.

[0079]

Additives miscible with tablets or capsules 25 include a binder such as gelatin, corn starch, tragacanth and gum arabic, an excipient such as crystalline cellulose, a swelling agent such as corn starch, gelatin and alginic acid, a lubricant such as magnesium stearate, a sweetening agent such as sucrose, 30 lactose and saccharin, and a flavoring agent such as peppermint, akamono oil and cherry. When the unit dosage is in the form of capsules, liquid carriers such as oils and fats may further be used together with the additives described above. A sterile composition for 35 injection may be formulated by a publicly known method

used to make pharmaceutical compositions, e.g., by dissolving or suspending the active ingredients in a vehicle such as water for injection with a naturally occurring vegetable oil such as sesame oil and coconut oil, etc. to prepare the pharmaceutical composition. Examples of an aqueous medium for injection include physiological saline and an isotonic solution containing glucose and other auxiliary agents (e.g., D-sorbitol, D-mannitol and sodium chloride) and may be used in combination with an appropriate dissolution aid such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol and polyethylene glycol), a nonionic surfactant (e.g., polysorbate 80TM and HCO-50), etc. As an oily medium, for example, sesame oil and soybean oil may be used, which can be used in combination with a dissolution aid such as benzyl benzoate and benzyl alcohol.

[0080]

Furthermore the prophylactic/therapeutic agent described above may also be formulated with a buffer (e.g., phosphate buffer and sodium acetate buffer) a soothing agent (e.g., benzalkonium chloride, procaine hydrochloride), a stabilizer (e.g., human serum albumin, polyethylene glycol), a preservative (e.g., benzyl alcohol, phenol), an antioxidant, etc. The thus prepared liquid for injection is normally filled in an appropriate ampoule.

Since the thus obtained preparation is safe and low toxic, it can be administered to human or mammals (e.g., rat, mouse, rabbit, sheep, swine, bovine, cat, dog, monkey, etc.).

The dose of the compound or a salt thereof varies depending on subject to be administered, target organ, symptom, method for administration, etc.; in oral

administration, the dose is normally about 0.1 to about 100 mg, preferably about 1.0 to about 50 mg, more preferably about 1.0 to about 20 mg per day for a patient with Alzheimer's disease (weighing 60 kg). In 5 parenteral administration, the single dose varies depending on subject to be administered, target organ, symptom, method for administration, etc. but it is advantageous to administer the active ingredient intravenously at a daily dose of about 0.01 to about 30 10 mg, preferably about 0.1 to about 20 mg, more preferably about 0.1 to about 10 mg for a patient with Alzheimer's disease (weighing 60 kg). For other animal species, the corresponding dose as converted per 60 kg weight can be administered.

15

[0081]

(7) Quantification of the receptor protein, the partial peptides or the salts of the present invention

20 The antibody of the present invention is capable of specifically recognizing the receptor protein of the present invention and accordingly, can be used for a quantification of the receptor protein of the present invention in a test sample solution, in particular, for 25 a quantification by sandwich immunoassay. Thus, the present invention provides, for example, the following methods for quantification:

(i) a method for quantification of the receptor protein or the like of the present invention in a test 30 liquid sample, which comprises competitively reacting the antibody of the present invention, a test liquid sample and a labeled receptor protein or the like of the present invention, and measuring the ratio of the labeled receptor protein or the like of the present 35 invention bound to said antibody; and,

(ii) a method for quantification of the receptor protein or the like of the present invention in a test liquid sample, which comprises simultaneously or continuously reacting the test liquid sample with an antibody of the present invention immobilized on an insoluble carrier and a labeled antibody of the present invention, and measuring the activity of the labeling agent on the insoluble carrier.

In the method (ii) described above, it is preferred that one antibody is capable of recognizing the N-terminal region of the receptor protein or the like of the present invention, while another antibody is capable of recognizing the C-terminal region of the receptor protein or the like of the present invention.

15

[0082]

The monoclonal antibody to the receptor protein or the like of the present invention (hereinafter sometimes referred to as the monoclonal antibody of the present invention) may be used to assay the receptor protein or the like of the present invention. Moreover, the receptor protein or the like of the present invention or the ligand peptide of the present invention can be detected by means of a tissue staining as well. For these purposes, the antibody molecule per se may be used or $F(ab')_2$, Fab' or Fab fractions of the antibody molecule may also be used. There is no particular limitation for the assaying method using the antibody to the receptor protein or the like of the present invention; any method may be used so far as it relates to a method in which the amount of antibody, antigen or antibody-antigen complex can be detected by a chemical or a physical means, depending on or corresponding to the amount of antigen (e.g., the amount of the receptor of the present invention) in a

test liquid sample to be assayed, and then calculated using a standard curve prepared by a standard solution containing the known amount of antigen. Advantageously used are, for example, nephrometry, competitive method,
5 immunometric method and sandwich method; in terms of sensitivity and specificity, the sandwich method, which will be described later, is particularly preferred.

Examples of the labeling agent used in the assay method using the labeling substance are radioisotopes,
10 enzymes, fluorescent substances and luminescent substances, etc. Examples of the radioisotope are [³H], [¹²⁵I], [¹³¹I], [³H], [¹⁴C], etc. Preferred examples of the enzyme are those that are stable and have a high specific activity, which include β -galactosidase, β -glucosidase, alkaline phosphatase, peroxidase and malate dehydrogenase. Examples of the fluorescent substance are fluorescamine, fluorescein isothiocyanate, etc. Examples of the luminescent substance are luminol, a luminol derivative, luciferin, lucigenin, etc.
15 Furthermore, a biotin-avidin system may also be used
20 for binding an antibody or antigen to a labeling agent.

[0083]

In the immobilization of antigens or antibodies,
25 physical adsorption may be used. Alternatively, chemical binding that is conventionally used for immobilization of proteins or enzymes may be used as well. Examples of the carrier include insoluble polysaccharides such as agarose, dextran and cellulose;
30 synthetic resins such as polystyrene, polyacrylamide and silicone; glass; etc.

In the sandwich method, a test sample liquid is reacted with an immobilized monoclonal antibody of the present invention (first reaction), then reacted with a
35 labeled monoclonal antibody of the present invention

(second reaction) and the activity of the labeling agent on the insoluble carrier is assayed, whereby the amount of the receptor protein of the present invention in the test sample liquid can be determined. The first 5 and second reactions may be carried out in a reversed order, simultaneously or sequentially with an interval. The type of the labeling agent and the method for immobilization may be the same as those described hereinabove.

10 In the immunoassay by the sandwich method, it is not always necessary that the antibody used for the labeled antibody and for the solid phase should be one type or one species but a mixture of two or more antibodies may also be used for the purpose of 15 improving the measurement sensitivity, etc.

In the method for assaying the receptor protein or the like of the present invention by the sandwich method according to the present invention, preferred monoclonal antibodies of the present invention used for 20 the first and the second reactions are antibodies, which binding sites to the receptor protein or the like of the present invention are different each other. Thus, the antibodies used in the first and the second reactions are those wherein, when the antibody used in 25 the second reaction recognizes the C-terminal region of the receptor protein, the antibody recognizing the site other than the C-terminal regions, e.g., recognizing the N-terminal region, is preferably used in the first reaction.

30

[0084]

The monoclonal antibody of the present invention or the ligand peptide of the present invention may be used in an assay system other than the sandwich method, 35 such as a competitive method, an immunometric method

and a nephrometry. In the competitive method, an antigen in a test solution and a labeled antigen are competitively reacted with an antibody, then an unreacted labeled antigen (F) and a labeled antigen 5 bound to the antibody (B) are separated (i.e., B/F separation) and the labeled amount of either B or F is measured to determine the amount of the antigen in the test solution. In the reactions for such a method, there are a liquid phase method in which a soluble 10 antibody is used as the antibody and the B/F separation is effected by polyethylene glycol while a second antibody to the antibody is used, and a solid phase method in which an immobilized antibody is used as the first antibody or a soluble antibody is used as the 15 first antibody while an immobilized antibody is used as the second antibody.

In the immunometric method, an antigen in a test solution and an immobilized antigen are competitively reacted with a given amount of a labeled antibody 20 followed by separating the solid phase from the liquid phase; or an antigen in a test solution and an excess amount of labeled antibody are reacted, then an immobilized antigen is added to bind an unreacted labeled antibody to the solid phase and the solid phase 25 is separated from the liquid phase. Thereafter, the labeled amount of any of the phases is measured to determine the antigen amount in the test solution.

In the nephrometry, the amount of insoluble sediment, which is produced as a result of the antigen- 30 antibody reaction in a gel or in a solution, is measured. Even when the amount of an antigen in a test solution is small and only a small amount of the sediment is obtained, a laser nephrometry utilizing laser scattering can be suitably used.

[0085]

- In applying each of those immunoassays to the assay method for the present invention, any special conditions or operations are not required to set forth.
- 5 The assay system for the receptor protein or the salt of the present invention may be constructed in addition to conditions or operations conventionally used for each of the methods, taking the technical consideration of one skilled in the art into account consideration.
- 10 For the details of such conventional technical means, a variety of reviews, reference books, etc. may be referred to (for example, Hiroshi Irie (ed.): "Radioimmunoassay" (published by Kodansha, 1974); Hiroshi Irie (ed.): "Radioimmunoassay; Second Series" (published by Kodansha, 1979); Eiji Ishikawa, et al. (ed.): "Enzyme Immunoassay" (published by Igaku Shoin, 1978); Eiji Ishikawa, et al. (ed.): "Enzyme Immunoassay" (Second Edition) (published by Igaku Shoin, 1982); Eiji Ishikawa, et al. (ed.): "Enzyme Immunoassay" (Third Edition) (published by Igaku Shoin, 1987); "Methods in Enzymology" Vol. 70 (Immunochemical Techniques (Part A)); ibid., Vol. 73 (Immunochemical Techniques (Part B)); ibid., Vol. 74 (Immunochemical Techniques (Part C)); ibid., Vol. 84 (Immunochemical Techniques (Part D: Selected Immunoassays)); ibid., Vol. 92 (Immunochemical Techniques (Part E: Monoclonal Antibodies and General Immunoassay Methods)); ibid., Vol. 121 (Immunochemical Techniques (Part I: Hybridoma Technology and Monoclonal Antibodies)) (published by Academic Press); etc.)
- 25
- 30

As described above, the receptor protein or the salt of the present invention can be quantified with high sensitivity, using the antibody of the present invention.

Furthermore, the receptor protein or the salt of the present invention can be quantified with high sensitivity, using the antibody of the present invention thereby to diagnose various diseases
5 associated with the dysfunction of the receptor protein of the present invention.

The antibody of the present invention can be employed for specifically detecting the receptor protein or the like of the present invention which may 10 be present in a test sample solution such as a body fluid, a tissue, etc. The antibody can also be used for preparation of an antibody column for purification of the receptor protein or the like of the present invention, detection of the receptor protein or the 15 like of the present invention in the fractions upon purification, and analysis of the behavior of the to the receptor protein or the like of the present invention in the cells under investigation.

20 [0086]

(8) Neutralization with the antibody to the receptor protein, the partial peptides or the salts of the present invention

The activity of the antibody to the receptor 25 protein, the partial peptides or the salts of the present invention that neutralizes the receptor protein or the like means the activity of inactivating the function of signal transduction in which the receptor protein participate. Therefore, when the antibody has 30 the neutralizing activity, the antibody can inactivate the signal transduction in which the receptor protein participate, for example, the receptor protein-mediated cell stimulating activities (e.g., the activities that promote or suppress arachidonic acid release, 35 acetylcholine release, intracellular Ca^{2+} release,

intracellular cAMP production, intracellular cGMP production, inositol phosphate production, changes in cell membrane potential, phosphorylation of intracellular proteins, activation of c-fos, and pH 5 reduction). Thus, the antibody can be used for the prevention and/or treatment of diseases caused by overexpression of the receptor protein.

(9) Preparation of animals containing the DNA encoding the G protein coupled receptor protein of the 10 present invention

Using the DNA of the present invention, transgenic animals that express the receptor protein or the like of the present invention can be prepared. Examples of the animals are mammals (e.g., rats, mice, rabbits, 15 sheep, swine, bovine, cats, dogs, monkeys) (hereinafter refer to as an animal) can be used, with particularly preferred being mice and rabbits.

To transfer the DNA of the present invention to a target animal, it is generally advantageous to use the 20 DNA in a gene construct ligated downstream a promoter capable of expressing the DNA in an animal cell. For example, when the rabbit-derived DNA of the present invention is transferred, for example, the gene construct, in which the DNA is ligated downstream a 25 promoter that can expresses DNA of the present invention derived from an animal which is highly homologous to the DNA of the present invention, is microinjected to rabbit fertilized ova. Thus, the DNA-transferred animal capable of producing a high level of 30 the receptor protein or the like of the present invention can be prepared. Examples of the promoter that can be used are a virus-derived promoter and a ubiquitous expression promoter such as metallothionein may be used but an NGF gene promoter and an enolase

gene promoter that are specifically expressed in the brain are used preferably.

[0087]

5 The transfer of the DNA of the present invention at the fertilized egg cell stage secures the presence of DNA in all germ and somatic cells in the target animal. The presence of the receptor protein or the like of the present invention in the germ cells in the
10 DNA-transferred animal means that all germ and somatic cells contain the receptor protein or the like of the present invention in all progenies of the animal. The progenies of the animal that took over the gene contain the receptor protein or the like of the present
15 invention in all germ and somatic cells.

The transgenic animal to which the DNA of the present invention is transferred can be subjected to a mating and a breeding for generations under common breeding circumstance, as the animal carrying the DNA,
20 after confirming that the gene can be stably retained. Moreover, male and female animals having the desired DNA are mated to give a homozygote having the transduced gene in both homologous chromosomes and then the male and female animals are mated so that such
25 breeding for generations that progenies contain the DNA can be performed.

The transgenic animal to which the DNA of the present invention is transferred is useful as the animal for screening of the agonist or antagonist to the receptor protein or the like of the present invention, since the receptor protein or the like of the present invention is abundantly expressed.

The transgenic animal to which the DNA of the present invention is transferred can also be used as
35 the cell sources for tissue culture. The receptor

protein or the like of the present invention can be analyzed by, for example, direct analysis of the DNA or RNA in tissues of the DNA-transferred mice of the present invention, or by analysis of tissues containing 5 the receptor protein expressed from the gene. Cells from tissues containing the receptor protein or the like of the present invention are cultured by the standard tissue culture technique. Using these cells the function of the cells from tissues that are 10 generally difficult to culture, for example, cells derived from the brain and peripheral tissues can be studied. Using these cells it is possible to select pharmaceuticals, for example, that increase the function of various tissues. Where a highly expressing 15 cell line is available, the receptor protein or the like of the present invention can be isolated and purified from the cell line.

[0088]

20 In the specification and drawings, the codes of bases and amino acids are denoted in accordance with the IUPAC-IUB Commission on Biochemical Nomenclature or by the common codes in the art, examples of which are shown below. For amino acids that may have the optical 25 isomer, L form is presented unless otherwise indicated.

DNA : deoxyribonucleic acid
cDNA : complementary deoxyribonucleic acid
A : adenine
T : thymine
30 G : guanine
C : cytosine
RNA : ribonucleic acid
mRNA : messenger ribonucleic acid
dATP : deoxyadenosine triphosphate
35 dTTP : deoxythymidine triphosphate

dGTP : deoxyguanosine triphosphate
dCTP : deoxycytidine triphosphate
ATP : adenosine triphosphate
EDTA : ethylenediaminetetraacetic acid
5 SDS : sodium dodecyl sulfate

[0089]

Gly : glycine
Ala : alanine
10 Val : valine
Leu : leucine
Ile : isoleucine
Ser : serine
Thr : threonine
15 Cys : cysteine
Met : methionine
Glu : glutamic acid
Asp : aspartic acid
Lys : lysine
20 Arg : arginine
His : histidine
Phe : phenylalanine
Tyr : tyrosine
Trp : tryptophan
25 Pro : proline
Asn : asparagine
Gln : glutamine
pGlu : pyroglutamic acid
Me : methyl group
30 Et : ethyl group
Bu : butyl group
Ph : phenyl group
TC : thiazolidine-4(R)-carboxamide group

Substituents, protecting groups, and reagents generally used in the specification are denoted by the codes below.

	Tos	: p-toluenesulfonyl
5	CHO	: formyl
	Bzl	: benzyl
	Cl ₂ Bzl	: 2,6-dichlorobenzyl
	Bom	: benzyloxymethyl
	Z	: benzyloxycarbonyl
10	C1-Z	: 2-chlorobenzyloxycarbonyl
	Br-Z	: 2-bromobenzyloxycarbonyl
	Boc	: t-butoxycarbonyl
	DNP	: dinitrophenol
	Trt	: trityl
15	Bum	: t-butoxymethyl
	Fmoc	: N-9-fluorenylmethoxycarbonyl
	HOBT	: 1-hydroxybenztriazole
	HOOBt	: 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine
20	HONB	: 1-hydroxy-5-norbornene-2,3-dicarboximide
	DCC	: N,N'-dichlorohexylcarbodiimide

[0091]

The sequence identification numbers (SEQ ID NO:) in the sequence listing of the specification indicates the following sequence, respectively.

[SEQ ID NO: 1]

The amino acid sequence of the rat cerebellum-derived novel G protein coupled receptor protein rOT7T175 of the present invention.

[SEQ ID NO: 2]

The base sequence of cDNA encoding the rat cerebellum-derived novel G protein coupled receptor protein rOT7T175 of the present invention containing the amino acid sequence shown by SEQ ID NO: 1.

[SEQ ID NO: 3]

The base sequence of primer 1 used for cloning the cDNA encoding the rat cerebellum-derived novel G protein coupled receptor protein rOT7T175 of the 5 present invention.

[SEQ ID NO: 4]

The base sequence of primer 2 used for cloning the cDNA encoding the rat cerebellum-derived novel G protein coupled receptor protein hOT7T175 of the 10 present invention.

[SEQ ID NO: 5]

The amino acid sequence of the human brain-derived novel G protein coupled receptor protein hOT7T175 of the present invention.

15 [SEQ ID NO: 6]

The base sequence of cDNA encoding the human brain-derived novel G protein coupled receptor protein hOT7T175 of the present invention containing the amino acid sequence shown by SEQ ID NO: 5.

20 [SEQ ID NO: 7]

The base sequence of probe used for cloning the cDNA encoding the human brain-derived novel G protein coupled receptor protein hOT7T175 of the present invention.

25 [SEQ ID NO: 8]

The base sequence of primer 1 used for cloning the cDNA encoding the human brain-derived novel G protein coupled receptor protein hOT7T175 of the present invention.

30 [SEQ ID NO: 9]

The base sequence of primer 2 used for cloning the cDNA encoding the human brain-derived novel G protein coupled receptor protein hOT7T175 of the present invention.

[0092]

Escherichia coli transformant DH10B/pAK-rOT175 obtained in Example 1 later described was on deposit with the Ministry of International Trade and Industry, 5 Agency of Industrial Science and Technology, National Institute of Bioscience and Human Technology (NIBH) as the Accession Number FERM BP-6553 on October 21, 1998 and with Institute for Fermentation, Osaka (IFO) as the Accession Number IFO 16209 on October 1, 1998.

10

[0093]

[EXAMPLES]

The present invention is described in detail below with reference to Examples, but not intended to limit 15 the scope of the present invention thereto. The gene manipulation procedures using *Escherichia coli* were performed according to the methods described in the Molecular Cloning.

20

[0094]

Example 1 Cloning of the cDNA encoding the rat cerebellum-derived G protein coupled receptor protein and determination of the base sequence
Using rat cerebellum cDNA as the template and two 25 primers, namely, primer 1 (SEQ ID NO :3) and primer 2 (SEQ ID NO :4), PCR reaction was performed. The reaction solution in the above reaction comprised of 1/10 volume of the cDNA for the template, 1/50 volume of Advantage cDNA Polymerase Mix (CLONTEC Inc.), 0.2 μ M 30 primer 1 (SEQ ID NO :3), 0.2 μ M primer 2 (SEQ ID NO :4), 200 μ M dNTPs and the buffer attached to the enzyme to make the final volume 50 μ l. In the PCR reaction, (1) the reaction solution was heated at 94°C for 2 minutes, (2) a cycle of heating at 94°C for 30 seconds followed 35 by 72°C for 2 minutes was repeated 3 times, (3) a cycle

of heating at 94°C for 30 seconds followed by 68°C for 2 minutes was repeated 3 times, (4) a cycle of 94°C for 30 seconds followed by 64°C for 30 seconds and 68°C for 2 minutes was repeated 30 times, and (5) finally, 5 extension reaction was performed at 68°C for 8 minutes. After completion of the PCR reaction, the product was subcloned to plasmid vector pCR2.1 (Invitrogen Inc.) following the instructions attached to TA cloning kit (Invitrogen Inc.), which was then introduced into 10 *Escherichia coli* DH5 α , and the clones containing the cDNA were selected on LB agar plates containing ampicillin. The sequence of each clone was analyzed to give the cDNA sequence (SEQ ID NO :2) encoding the novel G protein coupled receptor protein. The novel G 15 protein coupled receptor protein containing the amino acid sequence (SEQ ID NO :1) deduced from the cDNA was designated rOT7T175.

Plasmid pAK-rOT022L in which the cDNA (SEQ ID NO: 2) encoding the rat cerebellum-derived G protein 20 coupled receptor protein rOT7T175 of the present invention was subcloned was introduced into *Escherichia coli* DH10B according to a publicly known method to give the transformant *Escherichia coli* DH10B/pAK-rOT022L.

25 [0095]

Example 2 Cloning of the cDNA encoding the human brain-derived novel G protein coupled receptor protein HOT7T175 and determination of the base sequence
30 Cloning of cDNA was performed according to the instruction attached to GENE TRAPPER (Life Technologies Inc.). The probe (SEQ ID NO: 7) was biotinylated and followed by hybridizing with denatured human brain DNA library (SuperScript cDNA library, Life Technology 35 Inc.). The single-stranded gene obtained was amplified

using primer 1 (SEQ ID NO: 8) to be double-stranded DNA. The gene was electroporated into E. coli DH10B to obtain the transformants by using ampicillin resistance as an index. Electroporation was performed using E. coli Pulser (Bio Rad) at 1.8 kV of voltage. The transformants obtained were selected by colony PCR with probe (SEQ ID NO: 7) and primer 2 (SEQ ID NO: 9) and the transformant: E. coli DH10B/pCMV-hOT175 was obtained. The novel G protein coupled receptor protein containing an amino acid sequence deduced from the cDNA (SEQ ID NO: 5) designated hOT7T175. Colony PCR was performed as follows. The reaction solution in the above reaction comprised of 1/50 volume of Advantage cDNA Polymerase Mix (CLONTEC Inc.), 0.2 μM probe (SEQ ID NO: 7), 0.2 μM primer 2 (SEQ ID NO: 9), 200 μM dNTPs, 1/25 volume of DMSO and the buffer attached to the enzyme to make the final volume 10 μl. In the PCR reaction, (1) the reaction solution was heated at 94°C for 10 minutes, (2) a cycle of heating at 94°C for 10 seconds followed by 60°C for 10 seconds and 68°C for 1 minutes was repeated 25 times.

[0096]

[Meritorious effects of the invention]

The G protein coupled receptor protein of the present invention, its partial peptide or salts thereof as well as the polynucleotide coding for the same (e.g., DNA, RNA and derivatives thereof) can be used: (1) for determination of the ligand (agonist), (2) for preparing antibodies and antisera thereto, (3) for construction of the expression system of a recombinant receptor protein, (4) for development of the receptor-binding assay system using the expression system and screening of a candidate pharmaceutical compound, (5) for drug design based on comparison with structurally

similar ligand-receptor, (6) as a reagent for preparing a probe or a PCR primer in gene diagnostic, (7) for preparing a transgenic animal, or (8) as a prophylactic/therapeutic agent in gene therapy.

5

[0096]

[Sequence listings]

SEQ ID No: 1

10

[0097]

SEQ ID No: 2

[0098]

SEQ ID No: 3

15

[0099]

SEQ ID No: 4

[0100]

20 [BRIEF DESCRIPTION OF THE DRAWINGS]

FIG. 1 shows the base sequence of DNA encoding the rat cerebellum-derived novel G protein coupled receptor protein rOT7T175 of the present invention obtained in Example 1, and the amino acid sequence deduced from the 25 DNA sequence (continued to FIG. 2).

FIG. 2 shows the base sequence of DNA encoding the rat cerebellum-derived G protein coupled receptor protein rOT7T175 of the present invention obtained in Example 1, and the amino acid sequence deduced from the 30 DNA sequence (continued from FIG. 1, continued to FIG. 3).

FIG. 3 shows the base sequence of DNA encoding the rat cerebellum-derived G protein coupled receptor protein rOT7T175 of the present invention obtained in

Example 1, and the amino acid sequence deduced from the DNA sequence (continued from FIG. 2).

FIG. 4 shows the hydrophobic plotting of the rat cerebellum-derived G protein coupled receptor protein rOT7T175 of the present invention prepared based on the amino acid sequence shown in FIGS. 1 through 3.

FIG. 5 shows the base sequence of DNA encoding the human brain-derived novel G protein coupled receptor protein hAL7T024 of the present invention obtained in Example 2, and the amino acid sequence deduced from the DNA sequence (continued to FIG. 6).

FIG. 6 shows the base sequence of DNA encoding the human brain-derived G protein coupled receptor protein hAL7T024 of the present invention obtained in Example 2, and the amino acid sequence deduced from the DNA sequence (continued from FIG. 5, continued to FIG. 7).

FIG. 7 shows the base sequence of DNA encoding the human brain-derived G protein coupled receptor protein hAL7T024 of the present invention obtained in Example 2, and the amino acid sequence deduced from the DNA sequence (continued from FIG. 6).

FIG. 8 shows the hydrophobic plotting of the human brain-derived G protein coupled receptor protein hAL7T024 of the present invention prepared based on the amino acid sequence shown in FIGS. 5 through 7.

ABSTRACT

[Problems to be solved]

5 Providing of a novel G protein coupled receptor protein
useful for screening of agonist/antagonist

[Means for solving problems]

The rat cerebellum and the human brain-derived G
protein coupled receptor proteins, the partial peptide
10 or salts thereof, the nucleic acids encoding the
receptor protein and the derivatives thereof, nucleic
acids having an antisense sequence to a base sequence
encoding the receptor protein and the derivatives
thereof, a method for manufacturing the G protein
15 coupled receptor protein, a method for determining
ligands of the G protein coupled receptor protein, a
method/kit for screening compounds that alter the
binding property between ligands and the G protein
coupled receptor protein, a compound obtainable by the
20 screening or the salt thereof, and an antibody to the G
protein coupled receptor protein.

[Meritorious effects]

The rat cerebellum and the human brain-derived G
25 protein coupled receptor proteins or nucleic acids
encoding the same, and the derivatives thereof are
useful: (1) for determination of a ligand to the
receptor protein of the present invention (agonist),
(2) as an agent for the prevention and/or treatment of
30 diseases associated with dysfunction of the G protein
coupled receptor protein of the present invention, (3)
as a genetic diagnostic agent, (4) for quantification
of a ligand to the G protein coupled receptor protein
of the present invention, (5) for screening of a
35 compound (agonist, antagonist, etc.) that alters the

binding property between the G protein coupled receptor protein of the present invention and a ligand, (6) as a agent for the prevention and/or treatment of various diseases, comprising a compound (agonist, antagonist,
5 etc.) that alters the binding property between the G protein coupled receptor protein of the present invention and a ligand, (7) for quantification of the receptor protein, its partial peptide or salts of the present invention, (8) for neutralization by antibodies
10 to the receptor protein, its partial peptide or salts of the present invention and (9) for preparation of a non-human animal bearing the DNA encoding the G protein coupled receptor protein of the present invention.